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Cross-protection in Fostera™ PRRS vaccinated nursery swine against contemporary, heterologous porcine reproductive and respiratory syndrome virus (PRRSV) field isolates from different lineages

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Cross-protection in Fostera™ PRRS vaccinated nursery swine against contemporary, heterologous porcine reproductive and respiratory syndrome virus (PRRSV) field isolates from different lineages

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ABSTRACT

Porcine reproductive and respiratory syndrome virus (PRRSV) emerged worldwide approximately 25 years ago, and continues to be the most costly disease of modern swine production. The genetic diversity of PRRSV is one of the major hurdles encountered in attempts to reduce the effects of the virus through vaccines that provide broad cross-protection. The objective of this research is to examine the efficacy of a recently approved vaccine, Fostera™ PRRS, in growing pigs against separate challenges with heterologous PRRSV strains belonging to lineage 9 and lineage 1. The selected challenge viruses were isolated from recent PRRSV field infections in swine that originated in Iowa. The results of both studies indicate vaccine-induced immunity confers partial protection against the effects of heterologous PRRSV infection, as measured by reduced quantities of virus in serum, oral fluids and bronchoalveolar lavage fluid, and an increase in average daily gain in vaccinated pigs compared to non-vaccinated pigs challenged with the same virus. The two studies resulted in differing levels of protection as measured by severity of lung lesions and quantities of virus in tissues; these differences may be due to several factors, including variations in study design or in the degree of heterogeneity of the challenge viruses. The results show that Fostera™ PRRS vaccine can provide growing pigs with partial protection against heterologous challenge with currently circulating PRRSV. To improve prevention and control efforts, further investigations are needed into the cross-protection elicited by commercial PRRSV vaccines against contemporary, heterologous virus strains.

CHAPTER 1: INTRODUCTION

Background

Porcine reproductive and respiratory syndrome virus (PRRSV) is the most costly pathogen currently affecting the swine industry. Vaccines are commonly used in various programs designed to mitigate the impact of disease in growing pigs. Due to the genetic diversity of PRRSV, vaccines do not result in complete protection against challenge with heterologous viruses. The effectiveness of PRRSV vaccines can be evaluated by several methods, including increased average daily gain, decreased lung lesions, and decreased viral replication within the animal. In order to make significant progress towards decreasing the impact of PRRSV infection in swine, approved vaccines should elicit significant cross-protective immunity that prevents infection with and transmission of PRRSV strains representative of those present in current production settings.

Objective

The objective of this research was to determine the efficacy of a recently approved commercial modified live PRRSV vaccine, Fostera™ PRRS, against challenge with two field strains of considerable genetic diversity compared to the strain represented in the vaccine and belonging to different PRRSV lineages.

Thesis Organization

This thesis consists of an introduction, literature review, two original research chapters that will be submitted for publication, and a conclusion. Chapter 2 serves as a literature review of several topics regarding PRRSV that are pertinent to the study design

of research trials presented in later chapters. Topics include PRRSV emergence, importance to the industry, viral structure and pathogenesis, immune response, control strategies, vaccination, and a summary of published PRRSV challenge studies.

Chapters 3 and 4 present original research regarding the efficacy of a commercial PRRSV vaccine, Fostera™ PRRS. The author's role in each study included development of study design, the growth, selection and titration of viral isolates, execution of the animal studies, sample collection and necropsy of experimental animals and interpretation of results. Chapters are presented as prepared for publication. Chapter 5 consists of a short discussion of results and conclusions.

CHAPTER 2: LITERATURE REVIEW

Introduction

In the late 1980's and early 1990's outbreaks of infertility and respiratory disease of unknown origin were described in the swine populations of North America, Europe, and Asia. After being referred to by several different names (such as swine infertility and respiratory syndrome, porcine epidemic abortion and respiratory syndrome, and mystery swine disease), a consensus was reached that the clinical description would be known as porcine reproductive and respiratory syndrome (PRRS). A virus associated with the disease was first isolated in Europe in 1991 and designated as the Lelystad virus,[1] with a North American strain isolated shortly thereafter; collectively, the new pathogen became known as porcine reproductive and respiratory syndrome virus (PRRSV). A retrospective study found anti-PRRSV antibody positive serum in Canada as early as 1979.[2] This is the earliest seropositive report of PRRSV, suggesting the virus was present in the swine population for several years before reports of clinical disease; however, PRRSV was not detected in the seropositive samples.

As the name suggests, the clinical disease caused by PRRSV includes both reproductive and respiratory manifestations. The reproductive component of the disease is characterized by increased abortions in sows and decreased fertility in both sows and boars; the respiratory component of PRRSV infection is characterized by interstitial pneumonia and dyspnea in pigs of any age.[3,4] The severity of clinical signs apparent during PRRSV infection is often dependent upon immune status of the animal, the strain of the virus,[5,6] and the presence of viral co-infections (such as swine influenza) or

secondary bacterial infections (such as *Haemophilus parasuis*, *Pasteurella multocida*, and *Streptococcus suis*).[4] Despite the vast amount of research into the dynamics of PRRSV infection, other unidentified factors may play a role in disease severity.

A study in 2005 estimated that clinical disease attributable to PRRSV infection costs the United States (US) swine industry approximately $560 million annually due to increased mortality, decreased reproductive performance, and reduced feed efficiency; 88% of these costs were attributed to effects of the virus in growing pigs.[7] Using year 2000 survey results from the National Animal Health Monitoring System (NAHMS), the researchers found that nearly 45% of breeding females were in PRRSV-positive herds. The study did not take into account the cost of vaccination, biosecurity measures, monitoring of endemic herds, or the effect of subclinical disease. A more recent study in 2012 estimated the cost of lost productivity due to PRRSV at $664 million, with growing pigs accounting for 55% of the total economic loss.[8] The researchers used a survey of swine veterinarians to gather data on incidence of PRRSV as well as additional production costs that were not evaluated in the previous study. Based on the survey results, they estimated an additional cost of $477 million to US swine producers in animal health, biosecurity, and outbreak related expenses attributed to PRRSV.

PRRS Virus and Pathogenesis

Porcine reproductive and respiratory syndrome virus is a positive sense, single-stranded RNA virus belonging to the order *Nidovirales,* the family *Arteriviridae*, and the genus *Arterivirus*,[9] which also includes equine arteritis virus, lactate dehydrogenase elevating virus of mice, and simian hemorrhagic fever virus.[10] The PRRSV genome is

approximately 15 kb, and nine open reading frames (ORF) have been identified.[11] ORF1a and ORF1b make up a large portion of the genome and encode for various non-structural proteins (NSP) involved with viral replication and subgenomic transcription, including RNA-dependent RNA polymerase, while ORFs 2a, 2b, 3 and 4 encode for minor structural proteins found in the viral envelope.[11] Minor structural proteins may play a role in determining viral tropism through interaction with target cell receptors.

The major structural and envelope proteins of PRRSV are encoded by ORFs 5 through 7.[12] The first major envelope protein, GP5, is encoded by ORF5 and is the most highly variable protein of PRRSV.[13] Variability in GP5 may contribute to the lack of immune cross protection between virus isolates,[14] and the results of several studies suggest that antibodies against GP5 were the most effective at virus neutralization.[15,16,17] A second major envelope protein, the M protein, is encoded by ORF6[10] and is highly conserved across PRRSV strains.[18] Research suggests that GP5 and M protein may be involved in viral attachment to and internalization by macrophages,[19,20,21] and research has shown both proteins are vital components of the envelope; deletion of either ORF5 or ORF6 results in a failure of viral reproduction.[22] The nucleocapsid (N) protein is encoded by ORF7.[11] The N protein is the most immunogenic protein of PRRSV, with anti-N protein antibodies appearing as soon as 5 days after infection; however, these antibodies do not play a role in virus neutralization.[17,23]

Within the host PRRSV displays a predilection for monocyte derived cells, particularly those in the lung and lymphoid tissues. The virus displays a particular tropism for pulmonary alveolar macrophages,[24] both *in vitro* and *in vivo*. In addition, several cell lines derived from monkey kidney cells have been used to grow and study

PRRSV.[25,26] A cell surface molecule, heparan sulfate, has been shown to interact with the M/GP5 proteins on the PRRSV envelope.[19] This molecule is found on many cells and although not necessary for viral infection, it is thought to loosely adhere to and concentrate the virus on the cell suface.[27] The macrophage-restricted protein sialoadhesin has been shown to be necessary for virus attachment and internalization, through the binding of the M/GP5 proteins.[28,29,30] Release of the virus within the cell and viral uncoating has been shown to involve a transmembrane protein, CD163.[31,32] Once inside susceptible macrophages, several proteases are also involved in uncoating the virus.[33] Infection with PRRSV does not only affect macrophages, but can also induce apoptosis of nearby uninfected cells.[34] Infection also impairs macrophage function, which reduces basic immune physiologic mechanisms such as phagocytosis,[35,36] and can also induce apoptosis of infected cells.[37]

Direct contact with infected tissues or fluids from swine and aerosolization of the virus are the most common routes of PRRSV transmission. After initial infection and replication, viral particles are released from the cell and can readily spread throughout the body via the bloodstream. Viral replication markedly increases the amount of infectious virus, and replication persists in macrophages of the lung, tonsil, and lymphoid organs.[38,39] Although viremia is typically resolved after 28 days, viral RNA has been detected in serum up to 251 days post infection,[24,40] and in congenitally infected pigs for up to 228 days.[41] Persistence may be due to immune modulation through altered cytokine expression,[42] ineffective cell-mediated immune response,[23] shielding of virus and viral proteins within endosomes,[43] and/or antibody-mediated enhancement.[44] Once infection is

established the virus is shed in most bodily fluids/secretions: virus has been isolated from serum, semen, saliva, feces, urine, and nasal secretions.[4]

Clinical signs in PRRSV-infected growing pigs include fever, anorexia, dyspnea, and lethargy, and are usually more severe in younger pigs.[4] Gross lesions typically include a mottled-tan, non-collapsing lung with markedly enlarged lymph nodes throughout the body.[5,45] Microscopic lung lesions include lymphocytic and macrophagic interstitial pneumonia commonly accompanied by type 2 pneumocyte hypertrophy, necrotic macrophages, and cellular debris accumulations within alveoli.[46] Infection in pregnant sows is characterized by an increase in abortions, stillbirths, mummified fetuses, and infertility.[47] Pathologic lesions reported in sows or piglets from outbreaks of reproductive failure are inconsistent, non-specific, and/or absent. Clinical signs, gross and microscopic lesions can vary due to differences in the virulence of the PRRSV strains.[5,6]

Viral Classification and Diversity

European PRRSV strains are also known as type 1 PRRSV, with the Lelystad virus as the prototype strain. North American strains are known as type 2 PRRSV, with the prototype strain VR-2332. Despite disease emergence at nearly the same time, there is considerable genetic diversity both between and within the two genotypes. Genetic sequence homology between type 1 and type 2 PRRSV in various regions of the genome is 55-79%.[13,48,49,50] While diversity between the two types varies, it is common for strains within each genotype to demonstrate only 80-85% homology at ORF5. There are also significant differences in antigenicity between genotypes.[51] The large disparity in genetic and antigenic viral characteristics between genotypes suggests that the original virus

species underwent an initial divergence event, with each genotype acquiring considerable genetic diversity while being isolated to their respective continents and swine populations.[52]

Due to the genetic, antigenic, and pathogenic variability within genotypes several other classification methods have been developed in an attempt to more closely group similar PRRSV strains. One early method of virus classification, that was initially developed to differentiate vaccine strains from field strains, involves analysis of the restriction fragment length polymorphism (RFLP) of each virus strain.[53] Determination of the RFLP patterns involves enzymatic digestion of an amplified ORF5 fragment by a set of three restriction enzymes. Digestion with each enzyme can result in several possible patterns; the cut pattern for each enzyme is given a number, and a three-number RFLP code is assigned to the virus (for example, RFLP 1-7-4).

While this method is fairly convenient, it does not represent an accurate comparison of viral genotypes. The RFLP code is essentially a description of the ORF5 sequence, which is highly variable and has little predictive value regarding the phenotype, antigenicity, or pathogenicity of the virus. Furthermore, enzymatic digestion of ORF5 relies on specific genome sequences, which can change as the virus mutates during replication within pigs.[54] Using PRRSV strain VR-2332, researchers performed 13 serial passages of virus in pigs and analyzed the RFLP patterns of the resulting isolates.[55] Twenty percent of the recovered viruses had a different RFLP code than the initial challenge virus, despite a change to the ORF5 sequence of only 0.5-1.45%. The findings suggest that closely related viruses based on sequencing may have different RFLP codes. While the different enzymatic digestion patterns were initially few in number, an increase

in testing along with ongoing genetic mutation has led to an increase in the number of digestion patterns and the number of distinct RFLP codes.[52]

In response to the increasing number of available ORF5 genomic sequences and in an attempt to group similar viruses more accurately, a system was developed that classifies type 2 PRRSV strains based on their ORF5 sequence.[56] Researchers used over 8,000 ORF5 sequences available from GenBank to create nine distinct phylogenetic PRRSV lineages. This classification scheme resulted in groups of virus strains that were less than 11% diverse within lineages. Lineages 3 and 4 represent a small number of PRRSV sequences, all of which were found in Asia. Lineages 2, 6 and 7 are also relatively small and mainly contain virus isolates from the US.

The vast majority (>85%) of PRRSV isolates cluster in one of the four remaining lineages, each containing virus strains from around the world. Lineage 5 has relatively low intralineage diversity, and contains the historical isolate VR-2332 (the type 2 prototype strain). Lineage 8 contains the historical isolates SDSU-73 and JA-142. Lineages 1 and 9 contain the largest number of isolates, and also the highest intralineage diversity of the four large lineages. Lineage 1 contains the historical isolate MN-184.[56]

There are several mechanisms that may explain the vast amount of genetic diversity present in PRRSV. The primary culprit is thought to be error prone RNA polymerase and the lack of a corrective mechanism to ensure genomic integrity during replication. The rate of nucleotide substitution in PRRSV is the highest of all RNA viruses.[57] Genetic recombination has been shown to occur, and may play a significant role in viral diversity.[58,59] Host induced mutation using cytidine deaminase enzymes has also been considered a mechanism capable of driving genetic diversity in RNA viruses.[60]

Immune Response to Infection

While infection with PRRSV eventually induces protective immunity against the challenge strain, development of immune protection is slow and cross-protection between genetically diverse strains is generally incomplete and highly variable. Evidence suggests this may be due to a weak innate immune response by the target cell of PRRSV, the pulmonary alveolar macrophage. Viruses typically elicit the production of interferon after infection of a host cell, which increases the production of cytokines that work collectively to suppress viral protein synthesis and replication.[61] Significantly increased cytokine production, such as interferon-alpha and tumor necrosis factor-alpha, is a not a feature of early PRRSV infection,[42,62,63] and different field isolates have been found to have differing abilities to induce cytokine production.[64] Low amounts of interferon production may also lead to a lack of natural killer cell activation.[65] Decreased to absent cytokine production early in infection may play a part in the delayed humoral immune response as well as the prolonged course of infection. Several studies have shown an eventual elevation in various cytokine levels (interferon-gamma, tumor necrosis factor, and interleukin-10) within the lung or BALF collected from infected pigs, but the increase was not apparent until 7-10 days after infection.[66,67,68]

The humoral immune response to PRRSV can be detected within one week of infection. IgM antibodies to PRRSV are generated within 5-7 days of infection, with peak IgM levels observed 14-21 days post inoculation.[69] Anti-PRRSV IgG antibodies can be detected by 7-10 days post infection, peak at 14-28 days post inoculation,[17] and persist for up to 10 months.[70] Antibodies specific for individual viral proteins are produced but not in a consistent timeframe; in one study, IgM to NSP-2 and N-protein peaked at 7 days

post infection with an IgG peak around 35 days, while peak levels of IgM to GP5 were not detected until 3-4weeks post infection.[71] The IgG response to GP5 was also delayed, being first detected at 21 days and peaking at 35 days post infection, one to two weeks after the resolution of viremia. While serum neutralizing antibodies to GP5, GP4, and M protein have been reported, antibodies to GP5 are considered most effective at neutralizing virus.[17,72] The role of neutralizing antibody remains unclear; studies have detected neutralizing antibodies along with concurrent viremia.[3,73] In addition, several antibody kinetics studies have failed to detect neutralizing antibodies in PRRSV inoculated pigs.[69,74]

While antibody production towards infectious agents is generally regarded as helpful, antibody-dependent enhancement of PRRSV infection may occur. Macrophages display receptors that bind antigen-antibody complexes for phagocytosis. Low levels of anti-PRRSV antibody may enhance the association of viral particles with pulmonary alveolar macrophages, leading to increased viral uptake.[44] An *in vitro* study showed that serum containing antibodies to PRRSV actually enhanced infection of alveolar macrophages.[75]

The T-cell response to PRRSV infection is first apparent around 4 weeks after infection where studies have demonstrated an increase in cytotoxic T-cells in the lung[76,77] and an increase in helper T-cells in the blood at that time.[78] However, this is a delayed and relatively weak response compared to the cell-mediated immune response induced by other viruses.[79] In addition, PRRSV infection results in a down-regulation of major histocompatibility complex expression on dendritic cells, negatively affecting the efficiency of antigen presentation.[80] Protective immunity to homologous PRRSV is

predicated on memory cell induction. Large numbers of memory B cells are produced and can be found in many lymphoid tissues, particularly the spleen, tonsil, and sternal lymph node.[71] Re-challenge may result in significant protection without an anamnestic antibody response, despite the abundance of memory B cells.[71,81] The immunity produced by PRRSV infection is of long duration.[82] However, viral persistence within the animal may exceed the life span of commercial pigs.[41]

Protective immunity to heterologous PRRSV strains is highly variable due in part to the antigenic diversity of the virus strains. Exposure to one PRRSV strain does not usually confer complete protective immunity against another. In a multi-strain challenge study, only heterologous strains were detected in pigs after challenge; attenuated vaccination only provided complete protection against homologous strains.[83] However, research into cross protection has found variable levels of partial protection between virus isolates, characterized by a reduction in clinical signs, decreased pathologic lung lesions, lower levels of viremia and/or increased average daily gain.

Diagnostic Testing

Due to highly variable and non-specific clinical signs, using clinical parameters alone to diagnose PRRSV infection is potentially inaccurate, biased, and unreliable. Gross and histologic changes caused by respiratory PRRSV infection are also non-specific, and may be complicated by the presence of viral co-infections or secondary bacterial infections. Additional diagnostic testing is necessary to confirm PRRSV infection; several methods have been developed and are routinely used today. Timely and

accurate diagnosis is critical to the swine industry in order to limit the spread and control outbreaks of PRRSV.

Most diagnostics are centered on the detection of either anti-PRRSV antibodies or detection of the virus itself. Antibodies to PRRSV are readily detected in serum and oral fluids by several methods, including serum neutralization, fluorescent focus neutralization, and enzyme-linked immunosorbent assay (ELISA).[84,85] Antibody testing of serum merely detect animals that have been exposed to and induced an immune response to PRRSV. They are commonly used to monitor herds that produce PRRSV-negative breeding animals. Immunohistochemistry (IHC) staining of formalin-fixed tissue uses antibody to detect viral antigen within tissue,[86] which has the added benefit of associating the virus with characteristic microscopic lesions.

Virus isolation was initially the only way of detecting PRRSV, and has been shown to be possible using many types of tissue infected with PRRSV including serum, lung, and semen. One drawback of virus isolation is that it requires live PRRSV; if the virus present within the sample is not viable, the test will be falsely negative. With the advent of polymerase chain reaction (PCR) testing, both viable and non-viable PRRSV can be detected through amplification of viral specific RNA. One benefit of PCR is its high sensitivity and specificity, and various samples can be routinely tested; viral RNA is commonly detected in lung tissue, organs of the immune system (tonsil and lymph nodes), serum, oral fluids, and semen from infected pigs.[40,87,88,89] While PCR verifies the presence of viral RNA, the test does not discriminate between viable and non-viable PRRSV; it is simply a test for the presence or absence of PRRSV genetic material.

Sequencing of the whole viral genome as well as selected ORF segments has allowed for detailed genetic comparison and sorting of isolates into various classification schemes. Sequencing of ORF5 is the most common, and is the basis for classifying virus strains into lineages. Genome sequencing can also be used to determine if virus isolates are wild-type or vaccine-type strains, and may be used as an epidemiological tool to trace the spread and mutation rate of PRRSV within an endemic area.

Control Strategies

Due to the economic cost of PRRSV infection, considerable amounts of time and effort have been devoted to the development of various methods for the control and/or elimination of PRRSV. Prevention, control, and elimination of PRRSV depends on many factors, including the type of production system, management practices, flow of animals, exposure status of the site and the incoming animals, on-farm biosecurity, and goals of the operation. Vaccination against PRRSV is one component commonly used in several strategies aimed at either the control or elimination of PRRSV.

Control of PRRSV within an endemic herd begins with stabilizing the herd exposure to PRRSV. An endemic herd with widespread immunity to the resident PRRSV strain is more likely to avoid production losses. After the entire population is protected, preventing the introduction of a novel strain is of utmost importance. A phylogenetic study of field isolates found that the most common source of new virus in a PRRSV-positive herd was from the introduction of replacement animals carrying a new PRRSV strain.[90] Prevention of PRRSV introduction through the use of PRRSV-negative semen is crucial; currently most boar studs have strict testing protocols in place to ensure semen is

PRRSV-negative. Another method for controlling PRRSV-positive herds is to acclimatize gilts before they are bred and introduced into the primary herd. This method relies on exposing gilts to the strain of PRRSV currently circulating at their eventual breeding farm during the growing phase, in order to stimulate a protective immune response that will help prevent infection, virus transmission, and reproductive failure upon introduction into the breeding herd.

Elimination of PRRSV from infected herds can be costly, but has been accomplished through several methods.[91] One method includes serologic testing and removal of all seropositive animals.[92] Another involves the entire depopulation of a farm, disinfection of all facilities, and repopulation with PRRSV-negative pigs. Both of these methods result in the early removal of animals and loss of future production. A potentially less expensive method involves ceasing the introduction of new females for six months. Herd closure results in the exposure of all breeding females to the resident PRRSV strain and allows time for the development of protective immunity and eventual elimination of the virus. The lack of naïve, susceptible animals should result in a drastic reduction or elimination of the virus. Elimination methods rely on strict biosecurity to protect against the introduction of novel strains. The effectiveness of PRRSV elimination may be enhanced or adversely affected by many factors within an integrated production system.

Vaccination

Various vaccination protocols have been developed in an attempt to improve immune protection against infection and clinical disease. While vaccination routinely

elicits some level of humoral and cell mediated immune response (as measured by neutralizing antibody and detection of interferon-gamma secreting cells),[93] the effectiveness of vaccination at reducing the severity of or preventing clinical disease is highly variable. This variability is due in large part to genetic diversity between the vaccine and wild-type viruses.

Inactivated PRRSV vaccines stimulate a very weak cell mediated immune response in naïve pigs,[94,95] and have been shown to provide little to no protective immunity.[96] They can be used as a booster to modified live virus (MLV) vaccination or intentional exposure, but are generally regarded as ineffective.[97] Currently, there are no licensed commercial inactivated PRRSV vaccines in the US, but there are several available worldwide. While inactivated vaccines have limited practical value on their own, they are considered safe with almost no chance of reversion to virulence. Recent research into nanoparticle-bound inactivated PRRSV has shown an increased efficacy based on the rate of virus clearance from serum and altered cytokine expression.[98]

In contrast, MLV vaccines consistently elicit an immune response, including both humoral and cell mediated immunity, which may result in protection against clinical disease.[14] Use of MLV vaccines has been shown to result in strain specific protective immunity,[83] reduced clinical disease,[99,100] and decreased viral shedding.[101] However, since the vaccine uses live virus the potential for reversion to virulence exists and does occur.[102] MLV vaccines have been shown to induce protective immunity against homologous challenge, but complete cross protection against heterologous strains has yet to be demonstrated. Differences in the vaccine strain used in various commercial

products along with diversity in regional circulating PRRSV strains may also contribute to the variability in effectiveness of MLV vaccination.

Several commercial MLV vaccines are currently available in the US. Ingelvac® PRRS MLV (Boehringer Ingelheim Vetmedica, Inc., St. Joeseph, MO) became the first approved PRRSV vaccine in 1994, and is labeled for use in healthy swine as an aid in the reduction of disease associated with both the reproductive and respiratory forms of PRRSV. The parental strain of the vaccine is the North American prototype PRRSV strain, VR2332. Both the vaccine and the parental strain belong to lineage 5.

Ingelvac® PRRS ATP (Boehringer Ingelheim Vetmedica, Inc., St. Joeseph, MO) is labeled for use as an aid in the reduction of disease associated with both forms of PRRSV, but is only recommended for use in PRRSV positive herds. It is a type 2 PRRSV vaccine belonging to lineage 8, along with its parental strain JA142. The label of both BIVI products claims a 4 month duration of immunity, based on internal research.

Prime Pac™ PRRS+ (Merck Animal Health, Summit, NJ) was approved in 2014 and is labeled for use as an aid in the reduction of clinical signs or reproductive disease and respiratory disease due to PRRSV. The vaccine is based on a field isolate, and the product label claims a 4 month duration of immunity, based on internal research.

Fostera™ PRRS (Zoetis Animal Health US, Florham Park, NJ) is an MLV vaccine recently approved and currently licensed in the US and Canada. It was originally labeled for use in healthy swine three weeks of age or older in PRRSV-positive herds, or in herds deemed at risk of exposure, as an aid in preventing clinical respiratory disease caused by type 2 PRRSV. It was recently licensed for use in one-day old swine, as well as sows and gilts as an aid in preventing reproductive disease. Fostera™ PRRS is the first

PRRSV vaccine to earn the claim "aid in preventing" respiratory disease. The company claims a 6 month duration of immunity, based on internal research. The vaccine is based on the type 2 PRRSV strain P129, which belongs to lineage 8.

Several novel approaches to vaccine development have recently been attempted. The effects of various adjuvant additions to a commercial MLV vaccine showed that IL-12 enhanced cell mediated immunity but did not reduce clinical signs.[103] Vectored vaccines using pseudorabies,[104] adenovirus,[105] and transmissible gastroenteritis virus[106] to express PRRSV proteins have been developed. Engineered chimeric vaccines combining a commercial MLV vaccine and a wild-type isolate have been investigated.[107] Several other vaccine platforms are under investigation, but unfortunately have yet to show improved efficacy over commercially available MLV vaccines.

Summary of PRRSV Vaccine Challenge Studies

The efficacy of PRRSV vaccines and the level of cross protection they confer have been studied ever since the virus was first isolated. The results of these studies can be very difficult to interpret, for several reasons. Vaccine selection and administration protocols, animal selection and housing, and the selection and measurement of various parameters involved in immune protection can be highly variable between studies. Challenge virus selection can vary depending on the research group. In an attempt to eliminate this discrepancy and allow comparison between research trials, many studies use historic isolates. Due to the vast amount of antigenic drift, historic isolates may be considerably different genetically and phenotypically from currently circulating field strains, rendering the results from these challenge studies of questionable significance

regarding vaccine performance against contemporary strains. In addition, many studies have looked at vaccine induced protection against homologous challenge without evaluating protection against heterologous strains of virus.

Early vaccine research focused on viral shedding and the prevention of reproductive failure due to PRRSV. In one study, sows given an inactivated PRRSV vaccine and challenged with the homologous type 1 PRRSV strain delivered higher numbers of live, healthy piglets compared to unvaccinated sows.[108] Another study found that boars vaccinated with an MLV vaccine had lower levels of viremia and shed less virus in semen than boars vaccinated with an inactivated vaccine.[109] The inactivated vaccine did not decrease the level or duration of viremia or the shedding of virus in semen when compared to the non-vaccinated control group. Another study evaluated the efficacy of MLV vaccination of boars against PRRSV challenge with the type 2 prototype strain VR-2332.[110] Vaccination reduced or eliminated the shedding of challenge virus in 4 out of 5 boars; however, semen quality was negatively affected both after vaccination and challenge.

Heterologous challenge studies using MLV vaccines that assess the effects of vaccination on respiratory disease have been reported. In one such study, two PRRSV field isolates from Japan were used to assess cross protection of a commercially available vaccine, Ingelvac PRRS MLV.[111] One challenge virus was 94.0% similar at ORF5 to the vaccine strain, and vaccination resulted in reduced virus titers in lung, lymph node, and bronchoalveolar lavage fluid (BALF). A significant reduction in the duration of viremia and magnitude of gross lung lesions was also reported. The second challenge isolate was 87.5% similar at ORF5, and vaccination had no significant effect on lung lesions or viral

titers in tissues or serum. However, these trials used field isolates from 2006, and compared data from two-to-four pigs per group in each study.

Cross-protection induced by MLV vaccines has been studied using a wild-type Lelystad-like PRRSV challenge strain (98% ORF 5 homology to the vaccine strain) and a wild-type Italian field challenge isolate from 2001 (84% ORF5 homology).[112] Pigs vaccinated with an MLV Lelystad-based vaccine were completely protected against challenge with the Lelystad-like virus at 49 days post vaccination, based on a lack of virus detection in serum and BALF. Those challenged with the Italian field isolate were partially protected based on lower virus titers in BALF and serum compared to unvaccinated pigs. Severity of lung lesions and virus levels in tissue were not assessed. These findings, along with those from the Japanese study, suggest that increasing diversity in the PRRSV genomic sequence at ORF5, when compared to the vaccine strain, may affect vaccine efficacy in regards to cross-protection.

Another recent study evaluated the efficacy of Ingelvac PRRSV MLV against both homologous (using the type 2 prototype strain VR-2332) and heterologous challenge (using a field isolate from Kansas).[113] The study reported a complete absence of viremia in the homologous challenge group at 7 days post challenge (dpc) along with significantly reduced lung lesions at necropsy; however, there was no significant decrease in viremia in the heterologous challenge group until 14 dpc. Pigs in the homologous challenge group also had significantly reduced lung lesions compared to the heterologous challenge group. While the study provides evidence for vaccine-induced protective immunity to homologous challenge, the level of protection was based on decreased lung scores despite the lack of negative controls for comparison. In addition, the heterologous

challenge virus used in the study was isolated in 2006 and data regarding ORF5 genomic sequence homology to the vaccine strain was not provided in the publication.

Another study looked at the benefit of vaccination using Ingelvac PRRSV MLV in a herd endemically infected with a homologous strain of the virus and the response to heterologous challenge after different vaccination schedules.[99] Historic PRRSV isolates were used as both the homologous (VR-2332) and heterologous (MN-184) challenge strains. Pigs were intentionally exposed to the homologous challenge virus, and then vaccinated according to various schedules. At 97 day after initial infection, pigs were challenged with the heterologous virus. The study found that exposure and vaccination reduced clinical signs and improved weight gain compared to unvaccinated pigs, but did not prevent infection with heterologous challenge virus. An assessment of lung lesions was not included in determining the level of protection. The study may be of limited value to field situations, as it would be uncommon to have a herd infected with a PRRSV that is homologous to the strain used in the vaccine.

A similar study investigated the benefit of Ingelvac PRRSV MLV in a herd endemically infected with a heterologous strain of the virus, along with the response to heterologous challenge.[100] A PRRSV field isolate was used to intentionally expose pigs, who were subsequently vaccinated according to various schedules. Ninety-seven days after initial infection with the endemic PRRSV, pigs were challenged with the historical isolate MN-184. Therapeutic vaccination through any of the three experimental protocols did not significantly reduce the viral load in tissue. However, the study found that previous PRRSV infection and vaccination resulted in partial protection against the heterologous MN-184 challenge, determined by a reduction in clinical signs, decreased

level of viremia, and improved growth performance. Evaluation of lung lesions and ORF5 sequence homology were not reported. The results suggest that a combination of previous exposure and vaccination to PRRSV can elicit partial protection against heterologous challenge.

Intranasal delivery of a commercially available MLV vaccine has been assessed with subsequent heterologous challenge.[114] The study found that intranasal vaccination of pigs with Ingelvac PRRSV MLV showed a reduction in clinical disease, decreased gross lung lesions, and a lower level of viremia at 15 days post inoculation (dpi), along with increased weight gain after heterologous challenge compared to unvaccinated pigs. However, the level of viremia was not significantly different at 30 or 60 dpi compared to non-vaccinated controls, and the historical isolate MN-184 was used as the challenge virus in this study.

The efficacy of experimental inactivated vaccines has recently been evaluated. In two separate studies, pigs administered an experimental homologous inactivated vaccine or a commercial attenuated vaccine demonstrated a significantly shortened viremic phase following challenge, while a heterologous inactivated and commercial inactivated vaccines had no effect on viremia.[115] While the results show some degree of partial protection against homologous challenge, the study reinforces previous study findings of the limited efficacy of inactivated vaccines compared to MLV vaccines at eliciting homologous protection, along with the complete lack of heterologous cross-protection induced by inactivated vaccines.

Another study evaluated the protective immune response in gilts elicited by vaccination with a commercial inactivated PRRSV vaccine based on a Lelystad-like

virus.[116] Two doses of vaccine were administered, and gilts were inoculated at 90 days gestation with an Italian field strain of PRRSV (no ORF5 percent homology was reported). Researchers found no significant difference in reproductive performance between vaccinated and unvaccinated gilts. In addition, the vaccine failed to prevent clinical signs and viremia; however, preweaning mortality was reduced, suggesting that even though the immune response produced through vaccination did not prevent infection, it may have conferred some protective benefit to the piglets. The authors suggest that inactivated vaccines could be used to prime the immune system to improve the response to a more virulent immunization/challenge later in life.

Since PRRSV genotypes are not confined to their respective continents and can be found together in mixed infections, a recent study in Asia evaluated potential cross-genotype protection of a commercially available type 1 PRRSV vaccine against a highly virulent type 2 field isolate.[117] Results of the study indicate partial protection, based on a reduction in clinical signs and an increase in weight gain in vaccinated pigs compared to unvaccinated pigs. No differences in viremia were found and vaccination did not induce neutralizing antibodies to the challenge strain, suggesting that cell-mediated immunity may play an important role in the partially protective effect MLV vaccination has on heterologous challenge using type 1 and type 2 PRRSV vaccination and challenge models.

Cell-mediated immunity was also reported as being responsible for cross protection in another study,[118] where researchers used a commercially available MLV vaccine and a heterologous field isolate that was only 84% homologous to the vaccine strain at ORF5. Vaccinated pigs showed significantly reduced clinical signs and increased

weight gain, but failed to show a difference in viremia when compared to controls. The study did not investigate differences in lung lesions or viral tissue distribution. In addition, despite only testing the vaccine against a single virus, the authors reported that their results supported a previous study[119] that suggested genetic differences or similarities between the challenge and vaccine viruses in the ORF5 sequence are not predictive of potential cross-protective immunity.

A recent field trial investigated various clinical disease and production parameters in Fostera™ PRRS vaccinated pigs compared to non-vaccinated pigs on three separate farms.[120] Field isolates recovered from the three farms were determined to range from 85.4-92.2% ORF5 homology; two of the isolates belonged to lineage 5 while the other was a lineage 1 PRRSV. Results showed vaccinated pigs had significantly lower days to market, higher average daily weight gain, decreased mortality, and reduced microscopic lung lesions. However, the farms in the study were selected simply due to the likelihood of PRRSV infection, the level of viremia was not significantly different between vaccinated and unvaccinated pigs, and no direct challenge of pigs with a known viral dose occurred.

Conclusion

Due to the differences in PRRSV strains, the virus's propensity for mutation, and the specificity of the immune response, heterologous challenge studies under controlled conditions would provide the best representation of the degree of cross protective immunity elicited by vaccination. Challenging vaccinated pigs with virus isolates currently circulating in swine would provide a better representation of potential vaccine

efficacy in commercial swine production than using historical isolates. In addition, research studies that challenge the same vaccine against several PRRSV of variable diversity may suggest ORF5 homology could be a useful tool in predicting the magnitude of potential cross-protection. To the knowledge of the author, there has been no heterologous challenge study reported that used a contemporary field isolate and demonstrated a reduction in gross and microscopic lesions, lower level of viremia, and an increase in average daily gain in vaccinated pigs.

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CHAPTER 3: EVALUATION OF CROSS-PROTECTION IN FOSTERA™ PRRS VACCINATED NURSERY SWINE CHALLENGED WITH A CONTEMPORARY, HETEROLOGOUS LINEAGE 9 PORCINE REPRODUCTIVE AND RESPIRATORYSYNDROME VIRUS

Prepared for submission for publication

Running title: Cross-protection in PRRSV vaccinated swine challenged with a lineage 9 virus.

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Abstract

Porcine reproductive and respiratory syndrome virus currently circulating in swine demonstrates marked genetic diversity and forms multiple phylogenetic lineages. Modified live PRRSV vaccines have been used in intervention strategies to provide protection against infection and reduce the impact of clinical disease. Prevention and control of the virus has become challenging due to antigenic differences observed in contemporary PRRSV isolated from swine. The objective of this study was to evaluate

the efficacy of Fostera™ PRRS vaccine administered to weaned pigs against challenge with a contemporary, heterologous lineage 9 PRRSV. The challenge virus 12-39404 was isolated from nursery pigs in 2012 and shared 92% ORF5 nucleotide homology with the Fostera™ PRRS vaccine virus. Magnitude of gross and microscopic lung lesions at 12 days post infection were significantly lower in pigs that received Fostera™ PRRS compared to the non-vaccinated and challenged group. Viremia at 7 and 12 days post infection was also significantly lower in pigs that received Fostera™ PRRS and challenge virus was detected at significantly lower quantities in lung and bronchoalveolar lavage fluid compared to non-vaccinated and challenged pigs at necropsy. Vaccinated pigs also demonstrated improved average daily gain during the post-vaccination period concurrent with mild respiratory clinical signs. These results suggest Fostera™ PRRS induced partial cross-protection against a heterologous PRRSV recently isolated from swine and may represent outcomes that could be expected in current field infections with a contemporary lineage 9 PRRSV.

Introduction

Porcine reproductive and respiratory syndrome (PRRS), characterized by reproductive failure or abortion in sows and respiratory disease in growing pigs, is caused by PRRS virus (PRRSV) that was initially isolated in the United States (US) in 1990.[1] PRRSV is a positive sense, enveloped, single-stranded RNA virus of the family *Arteriviridae* in the order *Nidovirales*.[2] The virus is ubiquitous in swine throughout the world and has been estimated to cost the US swine industry approximately $664 million annually.[3,4,5] Effects in growing pigs account for 55% of these losses, mostly attributed to

increased mortality and elevated feed costs due to decreased feed efficiency.[4] Infection with PRRSV commonly results in interstitial pneumonia, but has also been shown to have immunosuppressive effects leading to increased susceptibility to infection with other swine pathogens.[6]

Two predominant PRRSV genotypes circulate in swine and are recognized as the European type 1 (strain Lelystad) and the North American type 2 genotypes (strain VR-2332).[7,8] However, PRRSV currently circulating in US swine, regardless of genotype, are genetically and antigenically diverse. The virus has rapidly evolved through genetic mutation, recombination and redistribution throughout swine-producing regions.[5,9,10,11,12,13] Phylogenetic analysis has also classified PRRSV into nine lineages and multiple sublineages representing immense differences in the genetic content and potential antigenic properties of the virus.[5] Lineage 1 PRRSV includes the RFLP pattern 1-8-4 that became prevalent in the early 2000's. Lineage 8 and 9 PRRSV were responsible for several field outbreaks involving abortion storms described in 1996 and includes the NADC20 isolate.[5,14] Currently, swine producers are encouraged to monitor the genotype of PRRSV circulating in their herd as well as swine producing regions, to detect the introduction of a new, antigenically distinct PRRSV.

Modified live and inactivated PRRSV vaccines have been used for the prevention and control of the virus with the goal of reducing the number of susceptible animals in the breeding herd, facilitating the production of negative piglets and preventing respiratory disease and production losses in grow-finish swine.[15] Inactivated PRRSV vaccines are less efficacious compared to PRRS modified-live virus (MLV) products.[16] Vaccination with PRRS MLV has demonstrated more effective control at reducing

clinical signs, decreasing lung lesions and viremia following challenge with homologous PRRSV; however, protection against heterologous infection is often inadequate.[17,18,19,20,21] A recent report demonstrated Fostera™ PRRS vaccinated pigs had increased average daily gain, decreased mortality and lower microscopic lung lesions compared to non-vaccinated swine when pigs were naturally exposed to the virus.[22] However, the study failed to show a significant decrease in viremia in vaccinated pigs and did not evaluate similar parameters using a heterologous challenge model under experimental conditions.

Experimental vaccine studies often use historical PRRSV isolates as challenge virus that may not be representative of the genetically diverse viruses currently circulating in the swine population.[20,23,24] Additional vaccine/challenge studies are needed using contemporary PRRSV that may represent potential cross-protection experienced under field conditions. Therefore, a lineage 9 heterologous PRRSV isolate was selected as the challenge inoculum from a field infection diagnosed in nursery pigs in 2012. The objectives of this study were to assess the magnitude of cross-protection induced by Fostera™ PRRS vaccine by comparing the level of viremia, gross and microscopic lung lesions, and average daily gain in vaccinated and non-vaccinated growing pigs challenged with a contemporary, heterologous lineage 9 PRRSV field isolate.

Materials and Methods

Animals

Sixty-two, four-week-old male, non-vaccinated weaned pigs were sourced from a single commercial, cross-bred farrow-to-wean herd. Upon arrival, all pigs were tested via

PCR for PRRSV and porcine circovirus type 2 (PCV2) in serum and influenza A virus (IAV) and *Mycoplasma hyopneumoniae* (MHP) in nasal swabs. Pigs were tested for the presence of antibody to PRRSV, IAV, PCV2, and MHP in serum prior to the start of the trial. All pigs were administered an intramuscular (IM) injection of ceftiofur crystalline free acid (Excede®, Zoetis, Florham Park, NJ) per label instructions upon arrival to the research facility and one day prior to challenge, and one IM injection of enrofloxacin (Baytril®, Bayer Healthcare, LLC, Shawnee Mission, KS) one day prior to vaccination to reduce potential mortality due to secondary bacterial infections common in swine.

PRRSV challenge virus

PRRSV isolate 12-39404A was recovered from serum collected from five-week-old nursery pigs located in Northwest Iowa in 2012. PRRSV 12-39404A was amplified by three passages on MARC-145 cells as previously described.[25] Infected cells were lysed by two freeze-thaw cycles after a PRRSV-specific cytopathic effect was observed. Clarification by centrifugation at 2000 x *g* for 10 min to pellet cell debris was followed by titration on MARC-145 cells, distribution of the virus in 5 ml aliquots and freezing at -80°C until challenge.

Experimental design

Pigs were block randomized by weight into three groups: non-vaccinated/non-challenged (NV/NC; *n*=21), non-vaccinated/challenged (NV/C; *n*=21), and vaccinated/challenged (V/C; *n*=20). Groups were housed in the same facility, but separated by room and ventilation system. Pigs in each room were fed a balanced diet *ad libitum* based on weight and given free access to water. At 0 days post vaccination (dpv)

pigs in the V/C group received a 2 ml intramuscular (IM) dose of Fostera™ PRRS vaccine per manufacturer's instructions. Pigs in the NV/NC and NV/C groups received a 2 ml IM dose of sterile diluent. At 28 dpv (0 days post inoculation (dpi)), pigs in the NV/C and V/C groups received 2 ml IM and 2 ml intranasal (IN) doses of challenge virus at $1x10^5$ $TCID_{50}$/ml. The NV/NC group received 2 ml IM and 2 ml IN doses of sterile cell culture medium. Serum was collected on 0, 14, and 28 dpv, and 3, 7, and 12 dpi. Pen-based oral fluids were collected on 0, 7, 14, 21, and 28 dpv, and 3, 7, and 12 dpi. Humane euthanasia was performed with a lethal dose of pentobarbital (Fatal-Plus, Vortech Pharmaceuticals, Ltd. Dearborn, MI) at 12 dpi. Fresh and formalin fixed samples collected at necropsy included: lung, tonsil, tracheobronchial, mediastinal, iliac, and inguinal lymph nodes. Five sections of formalin fixed lung were collected from the right cranial, right caudal, and the accessory lung lobes of each pig after insufflation and submersion for 15 minutes in 10% neutral buffered formalin. Broncho-alveolar lavage fluid (BALF) was collected from the left lung lobes at necropsy. The experimental design is described in Table 1 and was approved by the Iowa State University Institutional Animal Care and Use Committee (protocol log #10-13-7659-S).

Clinical assessment

Body weights were recorded for each pig on arrival, prior to challenge (25 dpv), and at necropsy (12 dpi). Weights were used for calculating average daily gain (ADG). Rectal temperature was recorded from -1 dpi through 7 dpi, and again at 10 dpi.

Pathology

At necropsy, lungs were evaluated for the presence of macroscopic lesions typical of PRRSV by a single veterinary pathologist blinded to the treatment groups. The percent of the lung surface affected with pneumonia was subjectively estimated for each lung lobe and the total percentage affected was calculated based on weighted proportions of each lobe relative to the total lung volume as previously described.[26] After 48 hrs fixation in neutral buffered formalin, tissue sections were trimmed, processed, and embedded in paraffin. Four micron thick sections were cut and stained with hematoxylin and eosin. All five lung sections were scored individually for interstitial pneumonia by a single veterinary pathologist blinded to the study, using the following scale: 0=normal, 1=mild multifocal, 2=mild diffuse, 3=moderate multifocal, 4=moderate diffuse, 5=severe multifocal, 6=severe diffuse. The scores were averaged to obtain an overall microscopic lung lesion score for each pig.

Differential Quantitative PCR

All PCR assays were conducted at the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL). Serum and oral fluid samples collected prior to challenge were extracted using the MagMAX™ Viral RNA Isolation Kit (Life Technologies, Carlsbad, CA) and a Kingfisher 96 instrument (Thermo Scientific, Waltham, MA) according to manufacturer's instructions. Real-time RT-PCR was performed on nucleic acid extracts using the commercially available MagMAX™ NA and EU PRRSV-specific PCR assay (Life Technologies Carlsbad, CA) to detect the

presence of Fostera™ PRRSV vaccine virus and ensure non-vaccinated pigs were virus negative.

Post-challenge serum, oral fluids, BALF, lung and tonsil were analyzed by a multiplex real-time RT-PCR with primers and probes designed at the ISU-VDL specifically targeting the challenge or vaccine virus. Three grams of fresh lung and tonsil were placed in 30 ml of MEM, processed by a tissue stomacher, and centrifuged to make a 10% homogenate. Samples were extracted using the MagMAX™ Viral RNA Isolation Kit (Life Technologies, Carlsbad, CA) and a Kingfisher 96 instrument (Thermo Scientific, Waltham, MA) according to manufacturer's instructions. Real-time RT-PCR was performed on nucleic acid extracts using the commercially available MagMAX™ NA and EU PRRSV-specific PCR assay (Life Technologies Carlsbad, CA) and substituting 400 nM of each forward and reverse primer and 200 nM of the probe specific to the challenge or vaccine virus (Table 2). Serum, lung and BALF reactions included 12.5 µl of 2X RT-PCR buffer, 2.5 µl of 10X PRRSV primer probe mix, 1.25 µL of 20X multiplex RT-PCR enzyme mix, and 0.75 µl of nuclease-free water. Each oral fluid reaction used the same volume of reagents described for the serum with the exception of 2.5 µL of 20X multiplex RT-PCR enzyme mix and 0.5 µL of nuclease-free water. A final volume of 25 µL consisting of 17 µL mastermix and 8 µL of RNA extract for the serum, lung and BALF samples or 18 µL mastermix and 7 µL of RNA extract for the oral fluid samples was placed in each well of a 96-well fast PCR plate (Life Technologies Carlsbad, CA). Real-time RT-PCR was performed using an AB 7500 fast thermocycler with the following cycling conditions: 1 cycle at 45°C for 10 min, 1 cycle at 95°C for 10 min, and 40 cycles of 97°C for 2 sec, 60°C for 40 sec. Amplification curves were

analyzed with commercial thermal cycler system software. Each PCR assay included eight progressive 1:10 dilutions of a known copy number of 12-39404A and Fostera™ PRRS vaccine virus based on genomic copies per ml. Aliquots of each dilution were stored at -80°C and used as the standard curve for quantitative RT-PCR.

Serology

All serology assays were conducted at the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) according to laboratory procedures. Serum from individual pigs and oral fluid samples from each pen post-vaccination and post-challenge were analyzed for the presence of anti-PRRSV antibody using the PRRSV X3 ELISA (IDEXX, Westbrook, ME).

Statistical Analysis

Percent pneumonia, lung lesion histopathology scores, $\log_{10}$ transformed PRRSV genomic copies in serum, lung, tonsil and BALF and PRRSV ELISA S/P ratios were analyzed using the Wilcoxon rank-sum test with a P-value ≤ 0.05 considered significant (SAS, SAS Institute, Cary, NC). Response variables shown to have a significant effect by treatment group were subjected to pair-wise comparisons using the Kruskal-Wallis test. Weight data was analyzed using analysis of variance (ANOVA). Response variables shown to have a significant effect by treatment group were subjected to pair-wise comparisons using the Tukey–Kramer test. A P-value ≤ 0.05 was considered significant.

Results

Pre- and post-screening Microbiological Assays

No extraneous viral or bacterial nucleic acid was detected in serum (PRRSV, PCV2) or nasal swabs (IAV, MHP) from pigs prior to the start of the experiment. All pigs were free of anti-PRRSV and -MHP antibodies prior to the start of the study. One of 62 pigs was positive for influenza A nucleoprotein antibody, and 27/62 pigs were positive for anti-PCV2 antibodies prior to the start of the experiment. BALF collected from all pigs at necropsy (12 dpi) tested negative for PCV2, IAV, and MHP nucleic acid.

Pre-challenge PRRSV ELISA and RT-PCR

All pigs in the V/C group were PRRSV ELISA antibody positive on 14 and 28 dpv with S/P ratios of 0.95 and 1.58, respectively (Figure 1). Fostera™ PRRS vaccine virus was detected in serum by PCR in 95% (19/20) and 85% (17/20) of pigs at 14 and 28 dpv, respectively. Pen-based oral fluid samples collected from the V/C group were positive for PRRSV ELISA antibody and Fostera™ PRRS vaccine virus at 7, 14, 21 and 28 dpv (data not shown). All pigs in the NV/NC and NV/C groups were PRRSV PCR and PRRSV antibody negative in serum and oral fluids during the pre-challenge phase of the study.

Clinical Disease

Pigs in the NV/NC group did not exhibit respiratory clinical signs for the duration of the study. Clinical signs of respiratory disease were not observed in the NV/C or V/C groups from 0 to 28 dpv. Pigs in the NV/C group became depressed, lethargic, and mildly

anorexic at 5 dpi, with a majority of pigs more severely affected at 7 dpi and clinical signs persisting through 12 dpi. The V/C group demonstrated mild lethargy from 7-10 dpi, although anorexia was not clinically apparent. Significant trends were not observed between the V/C and NV/C group mean body temperatures at any time point during the study (data not shown).

Lung Pathology

Macroscopic lesions in the NV/C and V/C group were characterized by mild to moderate enlargement of tracheobronchiolar lymph nodes and varying amounts of lung surface affected by mottled-tan pneumonia that were more extensive in the NV/C pigs (Figure 2). Mean percent pneumonia was significantly lower in the V/C group (mean 10.2%; range 0-40%) compared to the NV/C group (mean 16.9%; range 5-51%) at necropsy (Table 3). The NV/NC group averaged 2.0% pneumonia (range 0-13%). Microscopic lesions consisted of multifocal to diffuse, mild to severe lymphohistiocytic interstitial pneumonia (Figure 2). Microscopic interstitial pneumonia scores were significantly lower in the V/C group (mean 2.41; range 1.0-4.2) compared to the NV/C group (mean 3.13; range 1.4-5.4) (Table 3). The NV/NC group mean score was 1.57 (range 0.2-2.8).

Post-challenge PRRSV Quantitative RT-PCR

PRRSV virus was not detected by RT-PCR in the NV/NC group at any time during the study. Fostera™ PRRS virus was not detected by the differential, multiplex RT-PCR in the NV/C or V/C groups throughout the post-challenge period. Challenge

virus was detected in serum from all pigs in the NV/C and V/C groups at 3, 7 and 12 dpi (Table 4). Mean PRRSV $\log_{10}$ genomic copies/ml of serum from NV/C and V/C pigs was not statistically different at 3 dpi. At 7 and 12 dpi, mean PRRSV $\log_{10}$ genomic copies/ml were significantly lower in the V/C group compared to the NV/C pigs.

Oral fluid samples from NV/C and V/C groups were positive for challenge virus at 3, 7, and 12 dpi (Table 4). Mean PRRSV $\log_{10}$ genomic copies/ml of oral fluid from NV/C and V/C pigs were similar at 3 dpi, and demonstrated a lower trend in the V/C group at 7 and 12 dpi.

PRRSV was not detected in tissues from the NV/NC group at necropsy. Mean $\log_{10}$ genomic copies/ml of the challenge virus in lung homogenate and BALF were significantly lower in the V/C pigs compared to the NV/C group (Table 3). In contrast, the NV/C group had significantly lower amounts of challenge virus in tonsil homogenate compared to the V/C group (Table 3).

Post-Challenge PRRSV ELISA Antibody

All pigs in the V/C group were positive for PRRSV ELISA antibody in serum at 3, 7, and 12 dpi with mean S/P ratios of 1.82, 2.05, and 2.20, respectively. PRRSV ELISA antibody was also detected in pen-based oral fluid samples at 3, 7 and 12 dpi in the V/C group (data not shown). Post-challenge, 0/21, 18/21, and 21/21 pigs in the NV/C group were PRRSV ELISA antibody positive via serum at 3, 7, and 12 dpi, respectively (Figure 1). The NV/C group was PRRSV antibody negative via oral fluid at 3 dpi, and PRRSV ELISA antibody positive at 7 and 12 dpi (data not shown). All pigs in the

NV/NC group were PRRSV antibody negative via serum and oral fluids throughout the challenge period.

Production Parameters

A statistical difference was not observed in mean average daily gain (ADG) between NV/NC, NV/C and V/C pigs from vaccination until challenge. Post-challenge mean ADG was significantly higher in the V/C group (0.71 kg/day) compared to the NV/C group (0.58 kg/day) (Table 5). The post-challenge NV/C and V/C mean ADG was significantly lower compared to the NV/NC group (0.84 kg/day).

Discussion

Porcine reproductive and respiratory syndrome virus has spread globally since its emergence in the US and is the most economically significant pathogen affecting the swine industry worldwide.[4,27] Swine producers have implemented multiple programs to prevent the introduction of PRRSV into negative herds or eliminate entry of a variant virus into infected but PRRSV stable farms.[28,29] One of the primary objectives of an effective PRRSV control program at the herd level is to produce PRRSV negative piglets at weaning.[30] Procedures commonly used to achieve this outcome include gilt acclimation,[30] live virus inoculation or exposure,[31] and implementation of inactivated or modified live virus vaccines. Currently, MLV PRRSV vaccines have demonstrated more consistent efficacy compared to inactivated products and provide adequate protection against reinfection with homologous PRRSV[24,32] or reduce viral shedding.[33] However, protection against challenge with a heterologous PRRSV may vary or be

incomplete.[17,18,19,23,34,35,36] In addition, experimental studies often use historic PRRSV isolates for challenge, such as VR-2332, that may not represent the diverse ecology of PRRSV currently circulating in swine.[18,32,33]

The objective of the current study was to evaluate the efficacy of Fostera™ PRRS, a commercial MLV vaccine, against a heterologous lineage 9 virus considered representative of contemporary PRRSV circulating in swine. A phylogenetic analysis based on the ORF5 and using lineage reference strains identified several recent lineage 9 isolates at the ISU VDL.[37] Isolate 12-39404 was chosen as the challenge virus after a pilot study in weaned pigs confirmed it was a virulent strain that induced macroscopic and microscopic lung lesions (data not shown). The 12-39404 challenge virus RFLP is 1-4-2 and the ORF5 nucleotide homology is 92.5% with Fostera™ PRRS and 91.5% homologous to the lineage 9 reference strain NADC20 (Figure 3). Whole genome sequencing determined 12-39404 is 92.6% and 82.6% homologous to the Fostera™ PRRS whole genome nucleotide and amino acid sequences, respectively. The challenge virus was considered sufficiently heterologous to the Fostera™ PRRS, which is based on the virulent lineage 8 US PRRSV isolate P129.

Fostera™ PRRS conferred partial cross-protection against clinical disease and infection in V/C pigs compared to the NV/C group that overall demonstrated more severe clinical signs although mortality was not observed in either challenge group. Mean percent pneumonia was 39.6% lower in the V/C group compared to the NV/C pigs (10.2% vs. 16.9%, respectively) and microscopic lesion scores were significantly lower in pigs that received Fostera™ PRRS vaccine. Challenge virus was detected at significantly lower levels in the lung and BALF from vaccinated pigs suggesting a

reduction in viral replication may have been responsible for the decreased severity of lung lesions compared to the NV/C group at necropsy. However, it is unknown why this trend was reversed in the tonsil where the quantity of challenge virus was higher in V/C pigs compared to the non-vaccinated group. The tonsil consists of lymphoid and connective tissue that may be more difficult to uniformly homogenize compared to the lung suggesting disproportionate quantities of the tissue may have falsely lowered the level of challenge virus detected in some of the NV/C pigs. PRRSV persists longer in tonsil and lymph nodes than serum and lung, up to 157 days under experimental conditions.[38,39] Challenged pigs in the current study were euthanized at 12 dpi to maximize the severity of lung lesions, which may have limited the time available to allow differences in PRRSV replication between the V/C and NV/C tonsil to be detected. These data are consistent with previous reports that describe tonsil as a source of virus during persistent infection.[40,41,42] However, even in the presence of humoral immunity, PRRSV asymptomatic infections have been shown to persist in tonsil and other lymphoid tissues, which may suggest a reason for the lack of differences in virus quantities observed in the tonsil from V/C and NV/C pigs in the current study.[38]

Although similar levels of 12-39404 were detected via qRT-PCR at 3 dpi in both challenge groups, PRRSV viremia was significantly reduced at 7 and 12 dpi in the V/C pigs. Pen-based oral fluid samples collected at 7 and 12 dpi also demonstrated lower levels of challenge virus in pigs that received MLV vaccine. These data suggest Fostera™ PRRSV vaccine reduced viremia in spite of the heterogeneity between vaccine and challenge viruses. In contrast, previous PRRSV MLV vaccine and challenge studies did not demonstrate significant reductions in viremia.[22,35,43] The reason for the discrepant

results with the current study remains unknown although the previous studies were conducted in naturally infected pigs where level of virus exposure may have varied among challenged pigs and genetic or antigenic differences between vaccine and challenge viruses may have varied between studies. PRRSV ELISA antibody was detected in Fostera™ PRRS vaccinated pigs at the time of challenge suggesting the immune response elicited by the vaccine contributed to a reduction in viremia. Interestingly, PRRSV fluorescent focus neutralizing (FFN) assays with Fostera™ PRRS antisera from pigs vaccinated longer than 42 dpi were conducted against the challenge virus and determined negative (data not shown) indicating a neutralizing antibody response specific to the 12-39404 PRRSV was not induced by Fostera™ PRRS or present at the time of challenge. It is unknown which component of the immune response may have contributed to partial protection or if biological factors contributed to the difference in level of viremia. The properties that induce protection after infection with live virus have not been fully elucidated.[15] However, differences in level of viremia observed between the Fostera™ PRRSV vaccinated and non-vaccinated pigs suggests the immune response, humoral or cell-mediated, may have contributed to the partial-protection observed in the current study.

Economic losses due to PRRSV infections in nursery and grow-finish swine are attributed to poor production realized through decreased average daily gain, reduced feed efficiency and increased mortality.[4] Mortality was not observed in the current study; however, pigs euthanized at 12 dpi may have precluded the time necessary to experience death loss under the conditions of the study. In contrast, 12 dpi was sufficient to detect significant differences in average daily gain (ADG) among the negative control and two

challenge groups. Although vaccinated pigs demonstrated lower ADG from vaccination until challenge compared to the non-challenged pigs, differences were not significant. Post-challenge, V/C pigs gained 18.3% more body weight per day compared to the NV/C group. A previous study demonstrated similar production advantages in vaccinated pigs after heterologous challenge compared to the non-vaccinated, challenged pigs in spite of similar levels of viremia between the experimental groups.[36] The significantly elevated ADG recorded in the NV/NC pigs compared to the groups that received 12-39404 supports the pathogenicity of the challenge virus. However, the lower levels of virus detected in the lung and reduced viremia detected in Fostera™ PRRS vaccinated pigs may have improved pig performance compared to non-vaccinated pigs in spite of partial cross-protection. At necropsy, clinical signs (depression and lethargy) in the V/C group were resolving while the NV/C group appeared to be at peak severity. It is unknown if extending the length of the challenge period would have resulted in even greater disparity in ADG between the V/C and NV/C groups.

In summary, vaccination with Fostera™ PRRS resulted in partial cross-protection against a contemporary, heterologous lineage 9 PRRSV isolated from a recent infection in nursery pigs. While cross-protection was not complete, vaccination corresponded with decreased levels of 12-39404 in serum and virus replication in lung and BALF, reduced gross and microscopic lung lesions and increased ADG compared to challenged pigs that did not receive vaccination. Future studies are needed to evaluate cross-protection against PRRSV from different lineages that demonstrate greater heterogeneity or antigenic variation than what has been described in the current study, using contemporary viruses that represent potential field infections. Lengthening the challenge period to gain further

insight into the production benefits of Fostera™ PRRS vaccination may provide additional useful information. Prevention and control of PRRSV continues to challenge the swine industry and additional challenge studies using commercially available vaccines and contemporary isolates are necessary.

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Table 1. Experimental design

						Days post vaccination: dpv				Days post inoculation: dpi			
Group	***N***	**Age**	**Vaccine**	**Age**	**Challenge**	**0**	**7**	**14**	**21**	**0**	**3**	**7**	**12**
NV/NC	21	4 wks	Non-vaccinated	8 wks	Non-challenged	S, NS, OF	OF	S, OF	OF	S, OF	S, OF	S, OF	S, OF Necropsy
NV/C	21	4 wks	Non-vaccinated	8 wks	ISU-12-39404	S, NS, OF	OF	S, OF	OF	S, OF	S, OF	S, OF	S, OF Necropsy
V/C	20	4 wks	Fostera PRRSV	8 wks	ISU-12-39404	S, NS, OF	OF	S, OF	OF	S, OF	S, OF	S, OF	S, OF Necropsy

NV/NC: non-vaccinated/non-challenged; NV/C: non-vaccinated/challenged; V/C: vaccinated/ challenged; S: serum; NS: nasal swab; OF: oral fluid; BALF: bronchoalveolar lavage fluid

Table 2. Nucleotide sequences of the forward and reverse primers and individual probes for 12-39404A challenge PRRSV and Fostera™ PRRS vaccine viruses used in the real time RT-PCR for absolute quantification of PRRSV genomic copies.

Name	Sequence
PRRSV (forward primer)	5’-GTGCTCTGGCTGCGTTGA-3’
PRRSV (reverse primer)	5’-CGCCAGGACATGCAGTTCT-3’
12-39404A PRRSV (MGB probe)	5’-VIC-TTGCTTCATCATCAGGTTT-3’
Fostera™ PRRS Vaccine (MGB probe)	5’-FAM-TTGCTTCGTTATTAGGCTTG-3’

Table 3. Mean macroscopic pneumonia, microscopic lung lesion scores and quantitative RT-PCR values for challenge virus in lung, BALF and tonsil collected from NV/NC, NV/C and V/C pigs at 12 dpi ± standard error of the mean. Different letters within a column represent a statistical difference at $P \leq 0.05$.

	Lung Lesions		Quantitative PCR (Mean log 10 genomic copies/ml±SE)		
Group	Macroscopic percent pneumonia (Mean±SE)	Microscopic lung scores (Mean±SE)	Lung	BALF	Tonsil
NV/NC	2.0 ± 0.1^{a}	1.57 ± 0.20^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
NV/C	16.9 ± 2.3^{b}	3.13 ± 0.24^{b}	6.93 ± 0.08^{b}	7.23 ± 0.09^{b}	6.71 ± 0.08^{b}
V/C	10.2 ± 2.4^{c}	2.41 ± 0.20^{c}	5.71 ± 0.19^{b}	6.56 ± 0.19^{c}	7.04 ± 0.09^{c}

NV/NC, non-vaccinated-non-challenged; NV/C, Non-vaccinated-challenged; V/C, vaccinated-challenged

Table 4. Mean PRRSV 12-39404A log_{10} genomic copies/ml ± standard error of the mean for serum and pen-based oral fluid samples from NV/NC, NV/C and V/C pigs collected at 0, 3, 7 and 12 days post-inoculation (dpi). Different letters within a column are statistically different at $P \leq 0.05$.

	Serum (Mean log 10 genomic copies/ml±SE)			Oral Fluid (Mean log 10 genomic copies/ml±SE)		
Group	3 dpi	7 dpi	12 dpi	3 dpi	7 dpi	12 dpi
NV/NC	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00	0.00	0.00
NV/C	6.30 ± 0.12^{b}	6.93 ± 0.09^{b}	6.19 ± 0.15^{b}	4.91	5.23	4.95
V/C	6.10 ± 0.14^{b}	5.61 ± 0.19^{c}	3.87 ± 0.26^{c}	5.08	4.69	3.14

NV/NC, non-vaccinated-non-challenged; NV/C, Non-vaccinated-challenged; V/C, vaccinated-challenged

Table 5. Average daily gain reported in kilograms during the post-vaccination and post-challenge phase of the study in NV/NC, NV/C and V/C groups ± standard error of the mean. Different letters within a column are statistically different at $P \leq 0.05$.

	Average Daily Gain in Kilograms	
Group	Post-Vaccination (-3 to 25dpv)	Post-Challenge (25dpv to 12dpi)
NV/NC	0.49 ± 0.02[a]	0.84 ± 0.02[a]
NV/C	0.50 ± 0.01[a]	0.58 ± 0.03[b]
V/C	0.46 ± 0.02[a]	0.71 ± 0.03[c]

NV/NC, non-vaccinated-non-challenged; NV/C, Non-vaccinated-challenged; V/C, vaccinated-challenged

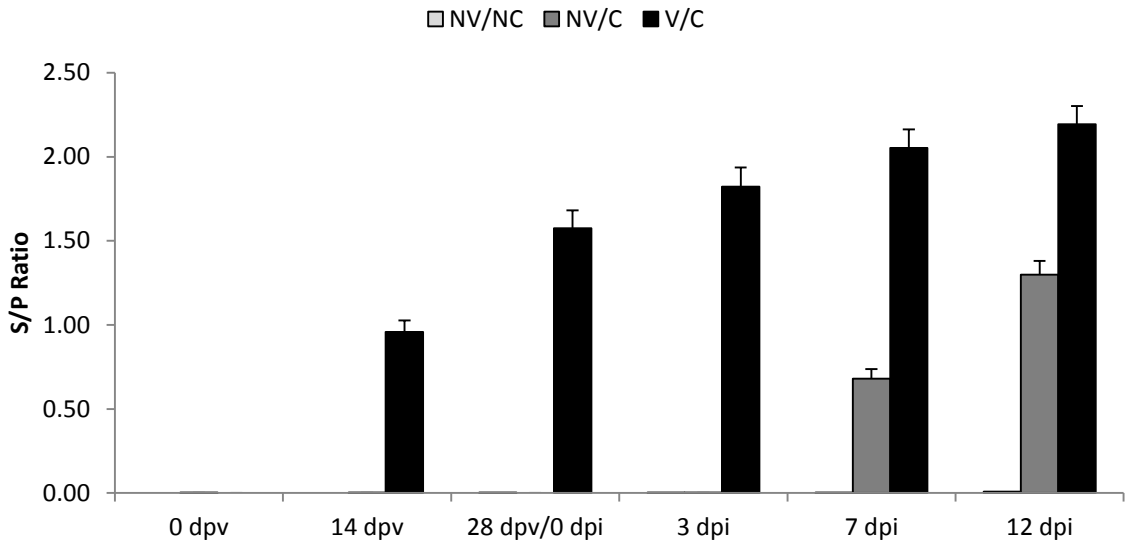

Figure 1. Mean anti-PRRSV ELISA antibody S/P ratios in serum from NV/NC, NV/C and V/C pigs at 0, 14 and 28 days post vaccination (dpv) and 3, 7 and 12 days post inoculation (dpi). Error bars represent standard error of the mean.

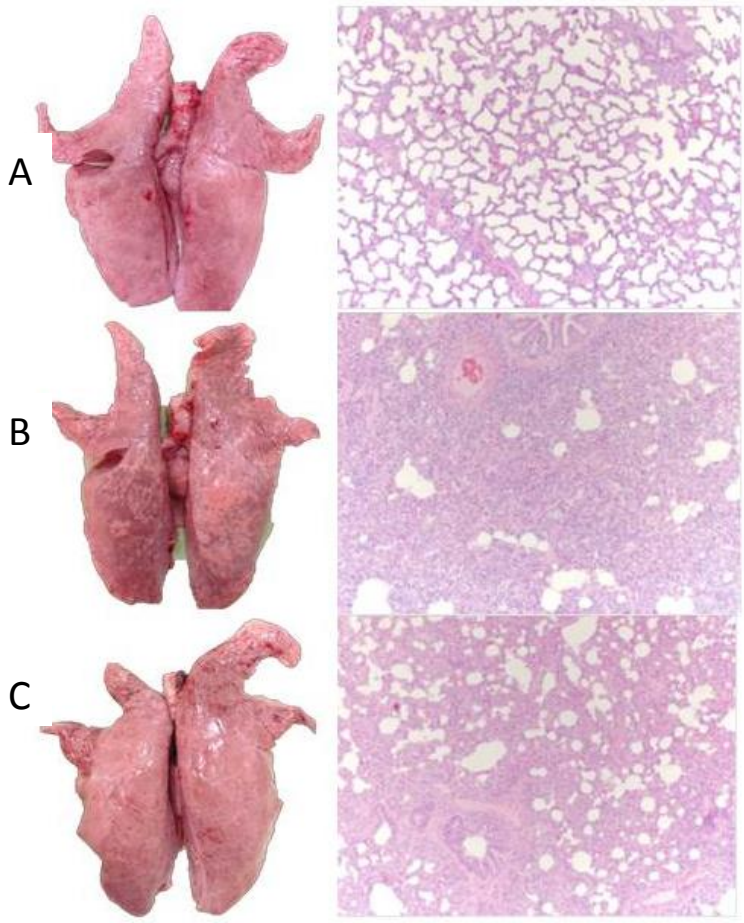

Figure 2. Macroscopic and microscopic lung lesions observed at necropsy in (A) NV/NC, (B) NV/C and (B) V/C pigs challenged with placebo or 12-39404 PRRSV.

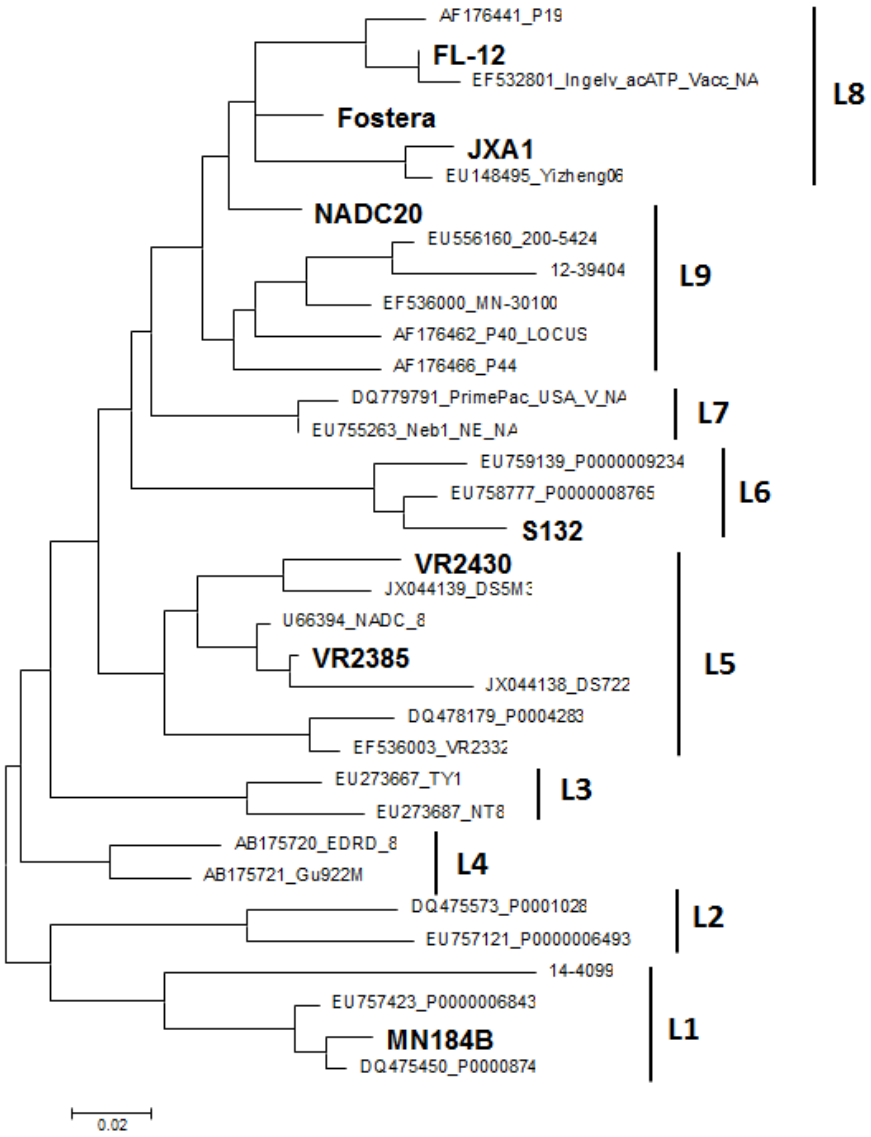

Figure 3. Phylogenetic analysis showing lineage classification and diversity, based on ORF 5 sequences, between historical reference PRRSV strains, several PRRSV strains used in vaccines, Fostera™ PRRS vaccine strain, and the PRRSV strain (12-39404) selected for challenge.

CHAPTER 4: EFFICACY OF FOSTERA™ PRRS VACCINE AGAINST A CONTEMPORARY, HETEROLOGOUS LINEAGE 1 PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS CHALLENGE IN NURSERY PIGS

Prepared for submission for publication

Running title: Cross-protection in PRRSV vaccinated swine challenged with a lineage 1 virus.

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Abstract

Porcine reproductive and respiratory syndrome virus is a diverse, ever-changing RNA virus that causes severe economic loss to the swine industry. Vaccination of pigs with modified live PRRSV vaccines have been used to control the impact of strains circulating within a herd, but have demonstrated variable protection against the introduction of antigenically diverse PRRSV. The objective of this study was to evaluate the efficacy of a commercial MLV PRRSV vaccine, Fostera™ PRRS, against challenge with a contemporary, heterologous lineage 1 virus. The challenge virus was isolated from serum collected from nursery pigs in Iowa in 2014, and shared 84.4% ORF5 nucleotide

homology with the vaccine strain. Viremia was significantly lower in vaccinated and challenged pigs at 3, 7, and 12 dpi, and average daily gain during the post-challenge period was significantly higher compared to the non-vaccinated and challenged pigs. Significantly lower levels of challenge virus were detected in bronchoalveolar lavage fluid from vaccinated pigs at necropsy. However, gross and microscopic lung lesion scores between vaccinated and non-vaccinated pigs were not significantly different. These data suggest that vaccination with Fostera™ PRRS elicited partial protection against a recently isolated, heterologous PRRSV lineage 1 virus demonstrated by the improved average daily gain and reduction in viremia in growing pigs challenged 28 days after vaccination.

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped, single-stranded RNA virus belonging to the Order *Nidovirales* and the family *Arteriviridae*.[1] Clinical disease results in reproductive failure and abortion in sows and respiratory disease in growing pigs; the clinical disease is commonly referred to as porcine reproductive and respiratory syndrome (PRRS). The classic pathologic lesion caused by viral infection in growing pigs is interstitial pneumonia; however, studies have shown that PRRSV infection can also result in increased susceptibility to co-infections with other viral and bacterial pathogens.[2] Recently, PRRSV was estimated to cost the United States (US) swine industry approximately $664 million each year, currently making PRRS the most economically important disease to the US swine industry.[3] The

economic effects of viral infection in growing pigs are the result of decreased feed efficiency and increased mortality.[3]

There are two recognized genotypes of PRRSV; European type 1 (reference strain Lelystad) and North American type 2 (reference strain VR-2332) and viruses in both genotypes display considerable genetic diversity. Type 2 PRRSV strains have been further classified into nine separate lineages based on phylogenetic analysis.[4] This classification system groups genetically similar PRRSV together, and as a result, the open reading frame 5 (ORF5) sequences within each virus lineage demonstrate no more than 11% nucleotide diverstiy.[4] The vast majority of PRRSV sequences fall into only 4 lineages (1, 5, 8 and 9). Classification of PRRSV strains is a helpful tool in epidemiological studies and may aid attempts to control disease, due to their high degree of genetic and antigenic diversity and the lack of cross-protection between strains elicited after natural infection. High rates of genetic mutation and recombination continue to allow PRRSV to evolve rapidly, and pose a challenge to the development of cross-protective vaccines.[4,5,6,7]

Both inactivated and modified live virus (MLV) PRRSV vaccines have been developed with the intent to stimulate broad cross-protection. Inactivated vaccines have been shown to be ineffective when used alone, but may potentially induce an anamnestic response after previous vaccination or natural infection. Modified live virus PRRS vaccines have been proven more efficacious at eliciting an immune response, and have demonstrated adequate protection against homologous challenge. Studies evaluating PRRSV MLV vaccines against heterologous challenge have found varying levels of partial protection based on a reduction in clinical signs and/or pathologic lung

lesions.[8,9,10,11,12] A recent field trial reported Fostera™ PRRS vaccination improved weight gain and decreased mortality and gross lung lesions; however, the trial did not use a heterologous virus or a standardized challenge dose of virus, and vaccinated pigs did not show differences in magnitude of viremia.[13]

While there are no commercial vaccines currently available that are based on a lineage 1 PRRSV, this genetic lineage encompasses the second highest number of virus strains with a published sequence.[4] Lineage 1 includes MN184 as the prototype virus and its variants characterized by various nucleotide deletions in the genome. The prototype virus was associated with a severe outbreak of PRRSV in the early 2000's.[14] Due to the prevalence of lineage 1 viruses and their potential for causing severe respiratory disease and abortion, vaccine induced cross-protection against this lineage is an important component of disease control and potential elimination in a single herd or in regional control efforts.

The objective of this study was to assess the efficacy of Fostera™ PRRS vaccine in growing pigs compared to non-vaccinated pigs after challenge with a lineage 1 PRRSV isolated in 2014. Viremia, gross and microscopic lung lesions, virus levels in lung, bronchoalveolar lavage fluid (BALF) and tonsil as well as average daily gain were evaluated.

Materials and Methods

Animals

Fifty-six, four-week-old male weaned pigs were sourced from a single commercial, cross-bred farrow-to-wean herd. Upon arrival, all pigs were tested via PCR

for PRRSV and porcine circovirus type 2 (PCV2) in serum and influenza A virus (IAV) and *Mycoplasma hyopneumoniae* (MHP) in nasal swabs. Pigs were tested at the Iowa State Univeristy Veterinary Diagnostic Laboratory for the presence of antibody to PRRSV, IAV, PCV2, and MHP in serum prior to the start of the trial and pigs were administered no vaccines prior to arrival. All pigs were administered an intramuscular (IM) injection of ceftiofur crystalline free acid (Excede®, Zoetis, Florham Park, NJ) per label instructions upon arrival to the research facility and one day prior to challenge, and one IM injection of enrofloxacin (Baytril®, Bayer Healthcare, LLC, Shawnee Mission, KS) one day prior to vaccination to control potential secondary bacterial infections.

PRRSV challenge virus

PRRSV isolate 14-4099 was recovered from serum collected from five-week-old nursery pigs located in Northwest Iowa in 2014 with clinical signs that included coughing, malaise and respiratory distress. PRRSV 14-4099 was amplified by three passages on MARC-145 cells as previously described.[15] Infected cells were lysed by two freeze-thaw cycles after a PRRSV-specific cytopathic effect was observed. Clarification by centrifugation at 2000 x g for 10 min to pellet cell debris was followed by titration on MARC-145 cells, distribution of the virus in 5 ml aliquots and freezing at -80°C until challenge.

Experimental design

Pigs were block randomized by weight into three groups: non-vaccinated/non-challenge (NV/NC; n=9), non-vaccinated/challenged (NV/C; n=24), and vaccinated/challenged (V/C; n=23). Groups were housed in the same facility, but

separated by room and ventilation system prior to challenge. Each group was divided into pens of two pigs, with two pens containing only one pig. Pigs in each room were fed a balanced diet *ad libitum* based on weight and given free access to water. At 0 days post vaccination (dpv) pigs in the V/C group received a 2 ml intramuscular (IM) dose of Fostera™ PRRS vaccine per manufacturer's instructions. Pigs in the NV/NC and NV/C groups received a 2 ml IM dose of sterile diluent. At 28 dpv (0 days post inoculation (dpi)) pigs in the NV/C and V/C groups received 2 ml IM and 2 ml intranasal (IN) doses of challenge virus at $1x10^5$ $TCID_{50}$/ml. The NV/NC group received 2 ml IM and 2 ml IN doses of sterile cell culture medium. In addition, pigs were co-mingled at challenge by randomly selecting one-half of the pigs in the NV/C and V/C groups, respectively, and moving them into the corresponding pens in the opposite rooms. Serum was collected from individual pigs at 0, 14, and 28 dpv, and 3, 7, and 12 dpi. Oral fluid samples were collected from each pen at 0, 7, 14, 21, and 28 dpv, and 3, 7, and 12 dpi. Humane euthanasia was performed with a lethal dose of pentobarbital (Fatal-Plus, Vortech Pharmaceuticals, Ltd. Dearborn, MI) at necropsy (12 dpi). Fresh and formalin fixed tissue samples collected at necropsy included: lung, tonsil, tracheobronchial, mediastinal, iliac, and inguinal lymph nodes. Five sections of formalin fixed lung were collected from the right cranial, right caudal, and accessory lung lobes of each pig after insufflation with and submersion in 10% neutral buffered formalin for 15 minutes for further fixation. Broncho-alveolar lavage fluid was collected from the left lung lobes at necropsy. The experimental design is described in Table 1 and was approved by the Iowa State University Institutional Animal Care and Use Committee (protocol log #10-13-7659-S).

Clinical assessment

Body weights were recorded for each pig on arrival, prior to challenge (26 dpv), and at necropsy (12 dpi). Weights were used for calculating average daily gain (ADG). Rectal temperature was recorded from -1 dpi through 7 dpi, and at 10 dpi.

Pathology

At necropsy, lungs were evaluated for the presence of macroscopic lesions typical of PRRSV by a single veterinary pathologist blinded to the treatment groups. The percent of the lung surface affected with pneumonia was subjectively estimated for each lung lobe and the total percentage affected was calculated based on weighted proportions of each lobe relative to the total lung volume as previously described.[16] After 48 hrs of fixation in neutral buffered formalin, tissue sections were trimmed, processed, and embedded in paraffin. Four micron thick sections were cut and stained with hematoxylin and eosin. All five lung sections were scored individually for interstitial pneumonia by a single veterinary pathologist blinded to the study, using the following scale: 0=normal, 1=mild multifocal, 2=mild diffuse, 3=moderate multifocal, 4=moderate diffuse, 5=severe multifocal, 6=severe diffuse. The scores designated for each section were averaged to obtain an overall microscopic lung lesion score for each pig.

Post-Challenge Quantitative PCR

All PCR assays were conducted at the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL). Serum and oral fluid samples collected prior to challenge were extracted using the MagMAX™ Viral RNA Isolation Kit (Life

Technologies, Carlsbad, CA) and a Kingfisher 96 instrument (Thermo Scientific, Waltham, MA) according to manufacturer's instructions. Real-time RT-PCR was performed on nucleic acid extracts using the commercially available MagMAX™ NA and EU PRRSV-specific PCR assay (Life Technologies Carlsbad, CA) to detect the presence of Fostera™ PRRSV vaccine virus and ensure non-vaccinated pigs were virus negative.

Post-challenge serum, oral fluids, BALF, lung and tonsil were analyzed by a real-time RT-PCR with primers and probes designed at the ISU-VDL specifically targeting ORF5 of the challenge virus. Three grams of fresh lung and tonsil were placed in 30 ml of MEM, processed by a tissue stomacher, and centrifuged to make a 10% homogenate. Samples were extracted using the MagMAX™ Viral RNA Isolation Kit (Life Technologies, Carlsbad, CA) and a Kingfisher 96 instrument (Thermo Scientific, Waltham, MA) according to manufacturer's instructions. Real-time RT-PCR was performed on nucleic acid extracts using the commercially available MagMAX™ NA and EU PRRSV-specific PCR assay (Life Technologies Carlsbad, CA) and substituting 400 nM of each forward and reverse primer and 200 nM of the probe specific to the challenge virus (Table 2). Serum, lung and BALF reactions included 12.5 µl of 2X RT-PCR buffer, 2.5 µl of 10X PRRSV primer probe mix, 1.25 µL of 20X multiplex RT-PCR enzyme mix, and 0.75 µl of nuclease-free water. Each oral fluid reaction used the same volume of reagents described for the serum with the exception of 2.5 µL of 20X multiplex RT-PCR enzyme mix and 0.5 µL of nuclease-free water. A final volume of 25 µL consisting of 17 µL mastermix and 8 µL of RNA extract for the serum, lung and BALF samples or 18 µL mastermix and 7 µL of RNA extract for the oral fluid samples

was placed in each well of a 96-well fast PCR plate (Life Technologies Carlsbad, CA). Real-time RT-PCR was performed using an AB 7500 fast thermocycler with the following cycling conditions: 1 cycle at 45°C for 10 min, 1 cycle at 95°C for 10 min, and 40 cycles of 97°C for 2 sec, 60°C for 40 sec. Amplification curves were analyzed with commercial thermal cycler system software. Each PCR assay included eight progressive 1:10 dilutions of a known copy number of 14-4099 challenge virus based on genomic copies per ml. Aliquots of each dilution were stored at -80°C and used as the standard curve for quantitative RT-PCR.

Serology

All antibody assays were conducted at the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) according to laboratory procedures. Serum from individual pigs and oral fluid samples from each pen post-vaccination and post-challenge were analyzed for the presence of anti-PRRSV antibody using the PRRSV X3 ELISA (IDEXX, Westbrook, ME).

Immunohistochemistry Staining

Immunohistochemistry (IHC) staining for the detection of PRRSV-specific antigen was performed on lung sections at the ISU-VDL as previously described.[17] Sections were scored for magnitude of immunoreactive signals by a single veterinary pathologist blinded to the study, using the following scale: 0=normal, 1=minimal, 2=mild, 3=moderate. Scores were averaged to obtain an overall treatment group mean for descriptive statistics.

Statistical Analysis

A statistical analysis was performed between the vaccinated/challenged and non-vaccinated/challenged groups. Descriptive summary statistics were performed for the non-vaccinated/non-challenged group. Percent lung lesions were transformed by the arcsine-square root transformation and were analyzed by a general linear mixed model approach. Microscopic lung lesions, virus levels in lung tissue, BALF, tonsil, oral fluids and serum and average daily gain were analyzed using the same statistical model. The SAS Proc Mixed procedure (SAS, Cary NC) included the fixed effect of treatment and the random effects of room, block-within-room and treatment-by-block-within-room. Treatment least squares mean (LS Mean) were calculated for each group. Pair-wise comparisons of LS Means between the two groups were performed by the two-sided Student's t-test at the 5% level of significance. A p-value ≤ 0.05 was considered significant.

Results

Pre- and Post-screening Microbiological Assays

No extraneous viral or bacterial nucleic acids were detected in serum (PRRSV, PCV2) or nasal swabs (IAV, MHP) from pigs prior to the start of the experiment. All pigs were free of anti-PRRSV and -MHP antibodies as well as influenza A nucleoprotein antibody prior to the start of the study. Anti-PCV2 antibody was detected in 54/56 pigs prior to the start of the experiment. BALF collected from all pigs at necropsy (12 dpi) tested negative for PCV2, IAV, and MHP nucleic acid.

Pre-challenge PRRSV ELISA and RT-PCR

Twenty-one of 23 and 23/23 pigs in the V/C group were PRRSV ELISA antibody positive at 14 and 28 dpv, respectively. Fostera™ PRRS vaccine virus was detected in serum by PCR in 87% (20/23) and 100% (23/23) of pigs at 14 and 28 dpv, respectively. All pigs in the NV/NC and NV/C groups were PRRSV PCR and PRRSV antibody negative in serum during the pre-challenge phase of the study.

Clinical Disease

Pigs in the NV/NC group did not exhibit clinical signs of respiratory disease for the duration of the study. Clinical signs of respiratory disease were not observed in the NV/C or V/C groups from 0 to 28 dpv. Approximately 75% of pigs in the NV/C group became depressed, lethargic, and moderately anorexic at 5 dpi; peak severity of clinical disease was apparent at 10 dpi characterized by moderate dyspnea, lethargy and marked anorexia. Approximately 50% of the V/C pigs displayed mild lethargy and anorexia from 5-12 dpi. Significant differences were not observed between the V/C and NV/C group mean body temperatures at any time point during the study (data not shown).

Lung Pathology

Macroscopic lesions in the NV/C and V/C group were characterized by moderate to marked enlargement of tracheobronchiolar lymph nodes and variable percentage of lung surface affected by mottled-tan pneumonia lesions consistent with PRRSV infection. Mean percent pneumonia was decreased in the V/C group (mean 12.63%; range 0-50%) compared to the NV/C group (mean 17.21%; range 2-58%); however, the difference in

gross lung lesions was not statistically significant (Table 3). The NV/NC group mean was 1.5% (range 0-12%).

Microscopic lung lesions consisted of multifocal to diffuse, mild to severe lymphohistiocytic interstitial pneumonia. Microscopic interstitial pneumonia scores were minimally decreased in the V/C group (mean 2.47; range 1.0-4.6) compared to the NV/C group (mean 2.57; range 0.8-4.6); however, the difference in microscopic lung lesions was not statistically significant. The NV/NC mean microscopic lung lesion score was 0.78 (range 0.4-1.0).

Post-challenge PRRSV Quantitative RT-PCR

PRRSV virus was not detected by RT-PCR in serum or oral fluid from the NV/NC group at any time during the study. PRRS challenge virus was detected in serum from all pigs in the NV/C and V/C groups at 3, 7 and 12 dpi. Mean PRRSV $\log_{10}$ genomic copies/ml of serum from V/C pigs was significantly lower compared to NV/C pigs at 3, 7, and 12 dpi (Table 4).

All pen-based oral fluid samples from NV/C and V/C groups were positive for PRRS challenge virus at 3, 7, and 12 dpi. Mean PRRSV $\log_{10}$ genomic copies/ml of oral fluid from V/C pigs was decreased compared to oral fluid from NV/C pigs at 3 and 7 dpi; however, the difference between groups was not statistically significant. Mean PRRSV $\log_{10}$ genomic copies/ml of oral fluid was significantly lower in V/C pigs at 12 dpi compared to NV/C pigs (Table 4).

PRRSV was not detected in tissues collected from the NV/NC group at necropsy. Mean $\log_{10}$ genomic copies/ml of the challenge virus in BALF was significantly lower in

the V/C pigs compared to the NV/C group (Table 3). Mean log10 genomic copies/ml of the challenge virus in lung homogenate was lower in V/C pigs compared to NV/C pigs but not statistically significant. In contrast, the V/C group had higher amounts of challenge virus in tonsil homogenate compared to the NV/C group; however, this difference was not significant.

Post-Challenge PRRSV ELISA Antibody

All pigs in the NV/NC group were PRRSV antibody negative via serum and oral fluids throughout the challenge period. All pigs in the V/C group were positive for PRRSV ELISA antibody in serum at 3 and 7 dpi with mean S/P ratios of 1.13 and 1.28, respectively (Figure 1). At 12 dpi, 21/23 pigs in the V/C group were positive for serum antibody to PRRSV, with a mean S/P ratio of 1.23. PRRSV ELISA antibody was also detected in pen-based oral fluid samples at 3, 7 and 12 dpi in the V/C group, with mean S/P ratios of 2.48, 4.01, and 4.41, respectively (data not shown). Post-challenge, 0/24, 21/24, and 24/24 pigs in the NV/C group were PRRSV ELISA antibody positive via serum at 3, 7, and 12 dpi, respectively. The NV/C group was PRRSV ELISA antibody negative via oral fluid at 3 dpi, and PRRSV ELISA antibody positive at 7 and 12 dpi, with mean S/P ratios of 0.97 and 4.21, respectively (data not shown).

Production Parameters

A statistical difference was not observed in mean average daily gain (ADG) between NV/C and V/C pigs from vaccination until challenge. Post-challenge mean ADG

was significantly higher in the V/C group (0.44 kg/day) compared to the NV/C group (0.32 kg/day) (Table 5). The post-challenge ADG in the NV/NC group was 0.83 kg/day.

Immunohistochemistry

Lung sections from all non-challenged control pigs were negative for PRRSV-antigen via IHC scoring. Mean lung section IHC scores for vaccinated and challenged pigs were 0.96 (range 0-3), while non-vaccinated and challenged pigs had a mean IHC score of 0.75 (range 0-3).

Discussion

Since its emergence in the late 1980's, PRRSV has become the most economically important disease of the swine industry. Vaccination against the virus may be utilized in various control programs to prevent the introduction of a new strain or aid in the production of PRRSV negative pigs at weaning. MLV vaccines have demonstrated more effective control compared to inactivated vaccines when challenged with a homologous PRRSV; however, MLV vaccines have only demonstrated the ability to induce partial protective immunity against heterologous challenge.[11] PRRSV vaccine challenge studies have typically used prototype isolates as the cell-culture challenge virus. Due to the large amount of genetic and antigenic diversity in PRRSV isolates, historical prototype viruses, such as VR2332 and MN-184, may not represent the genotypic or phenotypic characteristics of current strains circulating in swine that vaccine-induced immunity must protect against.

The objective of the current study was to evaluate the efficacy of a commercial MLV vaccine, Fostera™ PRRS, against a heterologous lineage 1 PRRSV recently isolated from nursery pigs in Iowa in 2014. Lineage 1 viruses are currently the most common PRRSV circulating in the US swine population demonstrating the greatest sequence divergence among field viruses.[18,19] Several recent lineage 1 virus isolates were identified from case submissions at the ISU VDL. Isolate 14-4099 was selected as the challenge virus after an initial pilot study confirmed its virulence in swine. Results of the current study suggest Fostera™ PRRS vaccine administered four weeks prior to challenge elicited partial cross-protection against a heterologous lineage 1 challenge virus that shares only 84.4% ORF5 nucleotide homology. Vaccinated pigs had significantly lower levels of viremia at all sampling points, and vaccinated pigs had lower levels of virus in oral fluids and BALF at 12 dpi. In spite of these differences in virus levels between vaccinated and non-vaccinated pigs, gross and microscopic lung lesions (while trending lower in vaccinated pigs) did not demonstrate significant differences between the challenge groups. It is unknown why significant differences in lung lesions were lacking between challenge groups, in spite of the differences in viremia. Previous studies suggest increasing genetic diversity between challenge and vaccine viruses may affect vaccine efficacy demonstrated by similar severity of lung lesions regardless of the vaccine administered.[12] PRRSV has a tropism for pulmonary alveolar macrophages (PAMs), which are the target of viral replication in swine.[20] Levels of challenge virus in BALF in the current study suggest viral replication was decreasing at 12 dpi although it is possible sufficient time was lacking for differences in viral clearance from lung and tonsil to be detected as suggested by the similar magnitude of IHC signals observed in lung

sections from both challenge groups, regardless of vaccination. Severity of lung lesions may also be influenced by the virulence of the challenge virus, dose, routes of administration and differences in host immune response. Collectively, pigs administered Fostera™ PRRSV demonstrated lower levels of virus in serum at all sample collections suggesting partial efficacy imparted by the vaccine.

The impact of co-mingling pigs in the same room may have also affected the magnitude of lung lesions observed in challenged pigs regardless of vaccination status. Research has shown that vaccinated boars shed lower amounts of virus in semen than non-vaccinated boars.[21] Studies in growing pigs have shown that therapeutic vaccination can decrease the amount of virus shed in herds endemically infected with homologous[8] or heterologous PRRSV strains.[22] In addition, experimental studies have reported the ability of infected pigs to transmit PRRSV to sentinel pigs via aerosols over short distances.[23] These studies suggest that co-mingling pigs could effectively expose vaccinated pigs to a greater dose of PRRSV throughout the post-challenge period (due to viral shedding from non-vaccinated roommates) compared to being exposed to pigs administered a similar treatment. While it is unclear what affect this may have on the dynamics of PRRSV infection, an artificial increase in virus levels detected in lung and elevated lung lesions are plausible outcomes.

In this study, each pig was given a standard dose of challenge virus. Oral fluids from vaccinated pigs contained lower amounts of virus than oral fluids from non-vaccinated pigs at 12 dpi, indicating that vaccination had resulted in lower quantities of virus being shed. Nose-to-nose contact would allow direct exposure to oral fluids, while aerosol transmission within each room also would be expected. Higher levels of

challenge virus shed from non-vaccinated pigs to vaccinated pigs may have contributed to the lack of difference in lung lesions or level of viral clearance in lung tissue; however, significantly lower levels of viremia and decreased PRRSV challenge virus in BALF from the vaccinated pigs would appear to contradict this theory. While the differences in serum and BALF virus quantities were still significant, the differences may have been falsely reduced from expected levels if pigs had not been co-mingled in the same room. Collectively, it could be argued that the co-mingling of vaccinated and non-vaccinated pigs post-challenge may affect the results of several commonly assessed parameters of immune protection.

Much of the losses attributed to PRRSV infection in growing pigs are due to increased mortality, decreased ADG, and decreased feed efficiency. In this study, a significant difference in average daily gain between vaccinated and non-vaccinated pigs was apparent after only 12 days post challenge with a virulent PRRSV. This suggests that vaccination with Fostera™ PRRS confers significant production benefits in the face of heterologous challenge. Considering clinical severity was at its peak at the time of necropsy in NV/C pigs, extending the post-challenge period may have further increased the difference in ADG between V/C and NV/C groups. In addition, vaccination did not depress ADG in the pre-challenge period, suggesting immunity was elicited without a concurrent decrease in production. The improved weight gain may be partially explained by the reduced viremia and reduced virus levels in BALF.

In summary, vaccination of nursery pigs with Fostera™ PRRS resulted in partial cross-protection when challenged with a contemporary, heterologous lineage 1 PRRSV. Vaccinated pigs showed decreased viremia and increased ADG compared to non-

vaccinated pigs; however, vaccination did not significantly decrease gross or microscopic lung lesions. Further investigation into cross-protection elicited by MLV vaccines is needed, along with research into the differences in cross-protection conferred by various commercial MLV vaccines. The results of this study show that the impact of PRRSV on growing pigs can be substantially mitigated through the use of MLV vaccines. In order to make substantial progress regarding PRRS control, future vaccine development should focus on the stimulation of broad heterologous cross-protection.

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Table 1. Experimental design.

							Days post vaccination: dpv				Days post inoculation: dpi			
Group	***N***	**Blocks**	**Age**	**Vaccine**	**Age**	**Challenge**	**0**	**7**	**14**	**21**	**0**	**3**	**7**	**12**
NV/NC	9	5	4 wks	Non-vaccinated	8 wks	Non-challenged	S NS OF	OF	S OF	OF	S OF	S OF	S OF	S, OF Necropsy
NV/C	24	12	4 wks	Non-vaccinated	8 wks	ISU-14-4099	S NS OF	OF	S OF	OF	S OF	S OF	S OF	S, OF Necropsy
V/C	23	12	4 wks	Fostera™ PRRSV	8 wks	ISU-14-4099	S NS OF	OF	S OF	OF	S OF	S OF	S OF	S, OF Necropsy

NV/NC: non-vaccinated/non-challenged; NV/C: non-vaccinated/challenged; V/C: vaccinated/ challenged; S: serum; NS: nasal swab; OF: oral fluid; BALF: bronchoalveolar lavage fluid

Table 2. Sequences of the forward and reverse primers and individual probe for 14-4099 challenge PRRS virus used in the real time RT-PCR for absolute quantification of PRRSV genomic copies.

Name	Sequence
PRRSV (forward primer)	5’-ATATCTCCACGGGCGGTATG -3’
PRRSV (reverse primer)	5’-TTGTAAGCCTGATGGCAAAGC-3’
14-4099 PRRSV	5’-/56-FAM/CTGTCTGCG/ZEN/CTCTTGCCGCG/3IABkFQ/-3’

Table 3. Mean macroscopic pneumonia, microscopic lung lesion scores and quantitative RT-PCR values in lung, BALF and tonsil collected from NV/NC, NV/C and V/C pigs at 12 dpi ± standard error of the mean. Different letters within a column represent a statistical difference at $P \leq 0.05$.

	Lung Lesions		Quantitative PCR (Mean log 10 genomic copies/ml±SE)		
Group	Macroscopic percent pneumonia (Mean±SE)	Microscopic lung scores (Mean±SE)	Lung	BALF	Tonsil
NV/NC	1.5 ± 0.7	0.78 ± 0.08	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
NV/C	17.2 ± 3.2[a]	2.57 ± 0.26[a]	6.21 ± 0.19[a]	6.19 ± 0.21[a]	6.71 ± 0.11[a]
V/C	12.7 ± 2.1[a]	2.47 ± 0.26[a]	6.04 ± 0.19[a]	5.44 ± 0.21[b]	6.87 ± 0.11[a]

NV/NC, non-vaccinated-non-challenged; NV/C, Non-vaccinated-challenged; V/C, vaccinated-challenged

Table 4. Mean PRRSV 14-4099 log_{10} genomic copies/ml ± standard error of the mean for serum and pen-based oral fluid samples from NV/NC, NV/C and V/C pigs collected at 3, 7 and 12 days post-inoculation (dpi). Different letters within a column are statistically different at $P \leq 0.05$.

	Serum (Mean log 10 genomic copies/ml±SE)			Oral Fluid (Mean log 10 genomic copies/ml±SE)		
Group	3 dpi	7 dpi	12 dpi	3 dpi	7 dpi	12 dpi
NV/NC	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
NV/C	6.92 ± 0.11[a]	7.07 ± 0.14[a]	5.38 ± 0.18[a]	4.83 ± 0.14[a]	5.12 ± 0.13[a]	4.36 ± 0.15[a]
V/C	6.54 ± 0.11[b]	5.78 ± 0.14[b]	4.18 ± 0.19[b]	4.76 ± 0.14[a]	4.86 ± 0.13[a]	3.53 ± 0.15[b]

NV/NC, non-vaccinated-non-challenged; NV/C, Non-vaccinated-challenged; V/C, vaccinated-challenged

Table 5. Average daily gain reported in kilograms during the post-vaccination and post-challenge phase of the study in NV/NC, NV/C and V/C groups ± standard error of the mean. Different letters within a column are statistically different at $P \leq 0.05$.

	Average Daily Gain in Kilograms	
Group	Post-Vaccination (-10 to 26dpv)	Post-Challenge (26dpv to 12dpi)
NV/NC	0.441 ± 0.028	0.827 ± 0.026
NV/C	0.405 ± 0.014[a]	0.323 ± 0.027[a]
V/C	0.405 ± 0.014[a]	0.441 ± 0.032[b]

NV/NC, non-vaccinated-non-challenged; NV/C, Non-vaccinated-challenged; V/C, vaccinated-challenged

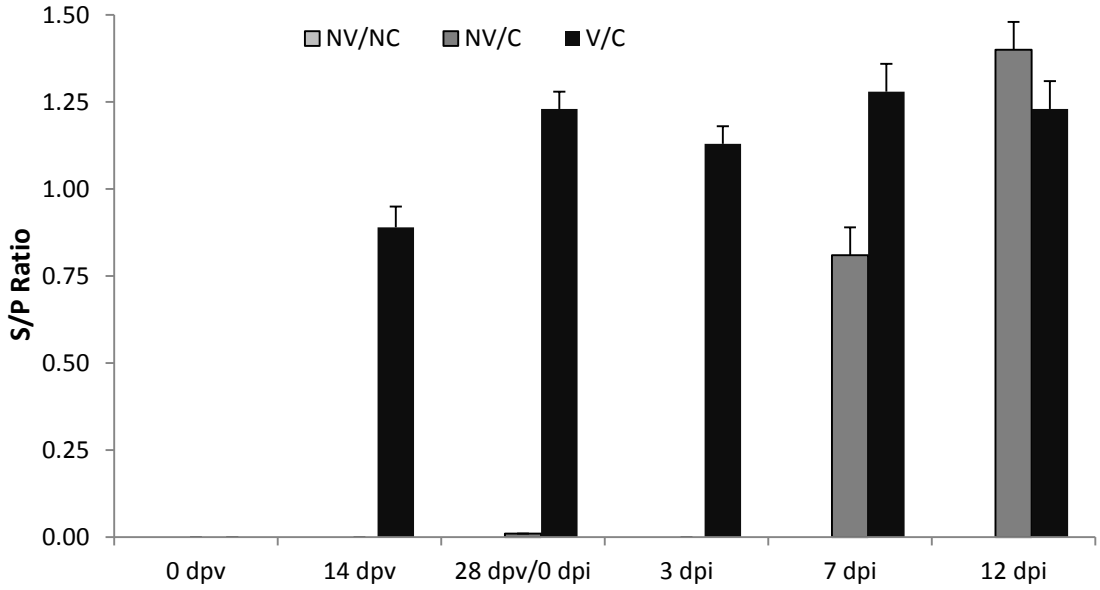

Figure 1. Mean anti-PRRSV ELISA antibody S/P ratios in serum from NV/NC, NV/C and V/C pigs at 0, 14 and 28 days post vaccination (dpv) and 3, 7 and 12 days post inoculation (dpi). Error bars represent standard error of the mean.

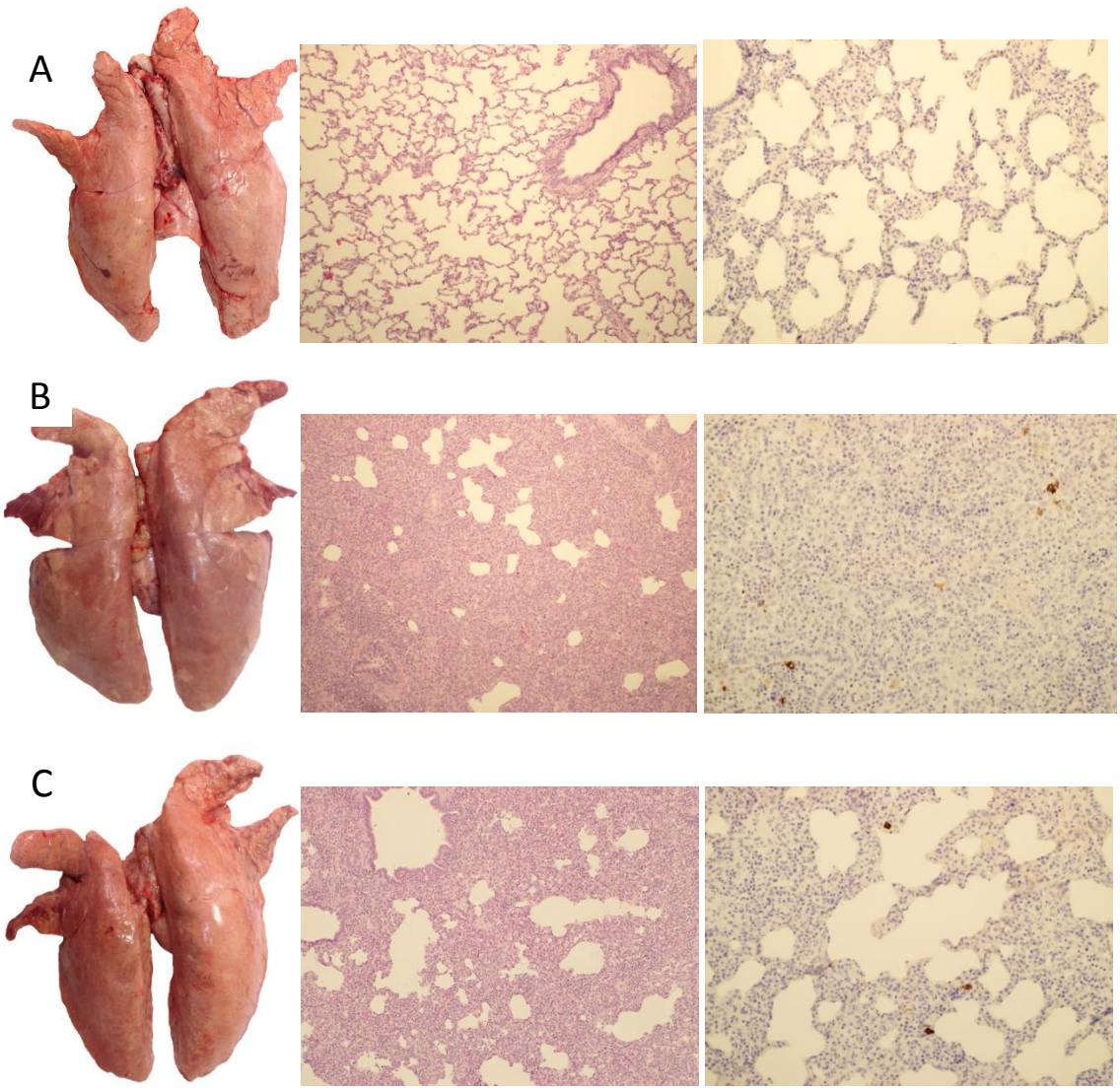

Figure 2. Macroscopic and microscopic lung lesions and immunohistochemistry staining of lung observed at necropsy in (A) NV/NC, (B) NV/C and (B) V/C pigs challenged with placebo or 14-4099 PRRSV.

CHAPTER 5: CONCLUSION

Reproductive failure, increased mortality, and reduced feed efficiency due to porcine reproductive and respiratory syndrome virus (PRRSV) combine to make it the mostly costly pathogen in modern swine production. Prevention and control of PRRSV is difficult due to the vast genetic diversity present within currently circulating virus strains. Protective immunity stimulated by natural infection or vaccination rarely results in complete protection from infection; however, vaccination with modified live virus (MLV) vaccines has been shown to result in partial protection, defined by an improvement in weight gain, less severe lung lesions, and/or reduced viremia.

The advancements made in vaccine development may play a crucial role in reducing the cost of disease, and someday may potentially result in PRRSV eradication. New vaccines must prove to be more effective than current commercially available vaccines, especially against relevant and contemporary field viruses. Most vaccine challenge studies have used historic PRRSV isolates in order to allow some level of comparison between results; however, historic isolates may be genetically and phenotypically different from current field strains and virulence among strains even within a PRRSV lineage may vary. A challenge model that uses currently circulating, virulent PRRSV isolates would provide a much more accurate depiction of how a vaccine could be expected to perform in current swine production settings.

With nine different lineages and thousands of available PRRSV isolates, the options for selecting a suitable challenge virus are nearly endless. One goal of the current research was to provide swine veterinarians and producers with information regarding a recently approved vaccine, Fostera™ PRRS. To ensure the results were relevant, the

following criteria for virus selection were as follows: 1) the challenge virus must belong to highly populated PRRSV lineage; 2) the challenge virus must be sufficiently heterologous to adequately evaluate cross-protection; and 3) the challenge virus must represent current circulating virus strains. Four of the nine lineage classifications have more than 1,000 different PRRSV. Lineage 1 and lineage 9 are the two largest PRRSV lineages; viruses within these lineages also display the most intralineage genetic diversity at ORF5 of any of the large lineages.

In both studies presented in this thesis, the challenge PRRSV were chosen from a group of recently isolated field isolates. Small pilot studies using three pigs in each challenge group were used to examine the virulence of each virus and to ensure the challenge model would result in gross lung lesions that were easy to observe. In addition to proving the virulence of the challenge virus, several different parameters were investigated, including microscopic lung lesions, average daily gain, and the amount of virus detected in various fluids or tissues (serum, oral fluids, bronchoalveolar lavage fluid, lung, and tonsil), to use as a measure of the degree of vaccine protection.

The results of the two studies presented here share several similarities. Average daily gain of vaccinated pigs during the challenge period was greater than that of non-vaccinated pigs in both studies, suggesting significant improvement in clinical disease that may be correlated with increased feed intake and improved feed efficiency although these parameters were not specifically measured during either experiment. Non-challenged controls had better average daily gain than non-vaccinated challenged pigs, which serves as a testament to the virulence of the challenge viruses; however, vaccinated pigs gained less weight than non-challenged controls as well, suggesting a level of partial

protection but with considerable room for improvement. Viremia was improved at 3, 7 and 12 days post inoculation and fewer viral genomic copies were also present in BALF at necropsy in both studies. Interestingly, both experiments detected higher levels of PRRSV in the tonsil tissue of vaccinated pigs. This unexpected result may be due to several factors, including processing difficulty due to the tough fibrous nature of the tissue, or perhaps priming the immune system via vaccination leads to enhanced viral uptake and replication in lymphoid tissue. PRRSV persists in lymphoid tissues of infected pigs even after clearance of the virus from the blood. However, it remains unknown why the vaccinated pigs demonstrated larger quantities of challenge virus in the tonsil compared to non-vaccinated and challenged pigs in the current studies.

While there were some similarities between the challenge experiments described in Chapter 2 and 3, there were also many differences between studies. The lineage 9 study demonstrated nearly a two log reduction in serum virus quantities in the vaccinated pigs at 7 and 12 dpi and no significant difference prior to that time. Vaccinated pigs also demonstrated lower levels of virus in lung tissue, along with significantly decreased gross and microscopic lung lesions. Challenge with a lineage 1 virus did not result in a decrease in lung lesions or virus load in the lung tissue, but did show a decrease in viremia at each measured timepoint (3, 7 and 12 dpi). The reasons for the discrepancies between studies are unknown; however, variation in study design (20 pigs per pen in the lineage 9 study and 2 pigs per pen with 12 replicates in the lineage 1 study), pig housing, and challenge virus along with differences in the individual pig's susceptibility and response to PRRSV infection may all play a role.

Along with random differences in the groups of pigs used in each study (although sourced from the same farm), two major differences are likely to have contributed to the variable results. First, the lineage 1 study used a challenge virus that was considerably more genetically diverse than the lineage 9 challenge virus regarding their homology to the vaccine PRRSV strain. Vaccines have been shown to be completely protective against homologous viruses used in challenge studies, so it stands to reason that a vaccine may offer a higher level of protection against a less genetically or antigenically diverse strain compared to a more diverse strain. The lineage 9 challenge virus shares 92.5% ORF5 homology with the vaccine virus, while the lineage 1 challenge virus is only 84.4% homologous at ORF5. This difference may account for the relatively higher degree of protection seen in the lineage 9 challenge study based on the reduction in lung lesions and reduced virus levels in lung tissue.

The second major difference involves the study design. The lineage 9 challenge study housed vaccinated and non-vaccinated pigs in separate rooms for the duration of the study (both pre-challenge and post-challenge periods). Pigs in each room were also kept in one large pen. The lineage 1 challenge study housed vaccinated pigs and non-vaccinated pigs in separate rooms for the pre-challenge period only, and pigs were divided into pens of two in each room. On the day of challenge, one-half of the pigs were moved into the opposite treatment room, in an attempt to eliminate potential room bias.

The design of the lineage 9 study eliminated any exposure to challenge virus between pigs from different treatment (vaccination) groups, and more accurately reflects what could be expected to occur in modern pig production when an entire group of pigs are administered the same vaccine at the same time. It allowed us to compare the

differences between an entire room of vaccinated pigs and an entire room of non-vaccinated pigs, to see if vaccination of a single room had any effect on post-challenge production. Each pig was individually vaccinated and challenged; during the post-challenge period, any additional virus contacted by the pig was due to the dynamics of infection and subsequent shedding of pen-mates within a treatment group, either with or without the potential benefit of vaccination. Room bias may have been present in the lineage 9 study, but in a climate-controlled infectious disease research facility with identical room dimensions, I believe the consequences to be insignificant and of irrelevant impact.

The design of the lineage 1 study removed room bias by co-mingling an equal number of pigs from each treatment group in each room, but in separate pens. Each pig was vaccinated and challenged individually; however, pigs in different treatment groups were allowed nose-to-nose contact. Therefore, at challenge, vaccinated pigs had direct contact with non-vaccinated pigs exposing vaccinated pigs to higher levels of PRRSV shedding into the environment compared to what would have been expected in a room containing pigs with the same treatment (vaccination vs. no vaccination). Conversely, with half of the pigs in each room vaccinated, non-vaccinated pigs came into contact with lower levels of virus. The study design effectively changed the exposure dose of each pig although it is unknown how that may have affected the effectiveness of vaccine-induced immunity in regards to parameters such as lungs lesions and weight gain.

The results of the studies presented here suggest vaccination provided partial protection as measured by a reduction in virus quantities in serum and increased weight gain. Interestingly, this protection was seen in the absence of neutralizing antibodies.

Serum from Fostera™ PRRS-vaccinated sows was tested for the presence of neutralizing antibodies toward 12-39404 (the challenge virus used in the lineage 9 challenge study) and showed a complete lack of neutralization. Antibody neutralization of the lineage 1 challenge strain, 14-4099, was not directly tested; however, due to the large amount of genetic diversity between this strain and the vaccine strain, neutralizing antibodies to a strain with that degree of diversity would not be expected. These results suggest that while neutralizing antibodies may be helpful (or even necessary) in preventing viremia, they are not needed to provide partial protection as measured by a reduction in viremia and improved production.

With the absence of complete protection from infection, the improvement in average daily gain observed in both studies is the most impressive aspect of vaccine-induced partial protection. The lineage 1 study ADG was particularly interesting, since no difference in gross or microscopic lung lesions were detected by a subjective analysis but based on expertise and published parameters. In a production setting with large numbers of animals, small increases in efficiency can have a large impact on the economics of disease. In addition to improved post challenge ADG, post vaccination average daily gain was not significantly different in either study. In other words, there are no negative effects on production due to vaccination with Fostera™ PRRS under the conditions of the experiments. In production systems with a high-risk of PRRSV exposure, producers can receive the benefit of partial protection without sacrificing production due to vaccination.

In order to eventually have any chance of eradicating PRRSV, science likely must develop a vaccine that confers complete (or near-complete) protection against heterologous infection. The rate of genetic change in field isolates will be a limiting

factor in the future effectiveness of current vaccines. More research is needed into highly conserved epitopes and antibodies to those epitopes that may provide broad cross-protection against current and future virus strains. There is a possibility that such epitopes may not exist, and vaccine development may need to explore new alternatives, such as nanoparticle or recombinant based vaccines.

In the meantime, more research is needed to determine the cross-protective efficacy of other commercial vaccines against challenge with PRRSV strains that are currently circulating in the world's swine population. Improving our knowledge of vaccine-induced immunity and its effect on various viruses can aid in the selection and directed use of the most appropriate vaccine for each production setting. Genetic sequencing of regional PRRSV strains may allow for widespread use of targeted vaccination programs in an effort to reduce the impact of PRRSV. Sequencing can also be useful in tracking the spread of the virus and identifying emerging routes of viral dissemination. Research into the various aspects of PRRSV has uncovered much of the mystery that initially surrounded the disease, but there is still much to learn.